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Professor C. D. DARLINGTON
Botany Department
Oxford

Sir RONALD FISHER
Whittingehame Lodge
Cambridge

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HEREDITY VOLUME 10 PART 3 DECEMBER 1956

FURTHER SELECTION EXPERIMENTS ON INDUSTRIAL MELANISM IN THE *LEPIDOPTERA*

Dr H. B. D. KETTLEWELL

Genetic Laboratories, Department of Zoology, University of Oxford

Received 22.iii.56.

1. PREVIOUS EXPERIMENTS

In a previous paper (Kettlewell, 1955), I recorded the results of extensive mark-release-recapture experiments undertaken in 1953 on the Peppered Moth, *Biston betularia* Linn. and its two melanic forms, *carbonaria* Jordan and *insularia* Th-Mieg (plate II, fig. 6). These experiments were carried out in a circumscribed area of woodland, the Christopher Cadbury Bird Reserve, situated about six miles from the industrial and heavily polluted area of Birmingham. The results then obtained may be summarised as follows :

(*a*) When released on to available trunks and boughs, their normal resting places, over 97 per cent. of *carbonaria* (the black form) appeared to the human eye to be inconspicuous. Conversely, nearly 89 per cent. of the light form of the Peppered Moth were adjudged conspicuous (plate I, fig. 2).

(*b*) Direct observation on the released insects showed that by late afternoon 54 per cent. of the light form had disappeared but only 37 per cent. of the *carbonaria*. Furthermore, we witnessed both Robins (*Erithacus rubecula* L.) and Hedge Sparrows (*Prunella modularis* L.) take the moths from off the trees, and they did this selectively and, on the majority of occasions, in an order of conspicuousness as previously scored by us.

(*c*) Recapture figures, reflecting a differential mortality rate, showed that more than twice as many *carbonaria* survived as *typical* (27·5 to 13 per cent.) (fig. 1*a*).

These experiments showed that birds act as selective agents and that the melanic forms of *betularia* are at a cryptic advantage in an industrial area such as Birmingham. It was essential to repeat these observations during a subsequent season and to extend them by carrying out comparable mark-release-recapture experiments in unpolluted countryside. This paper provides details of such work, which was undertaken in 1955.

2. A REPEAT OF THE SELECTION EXPERIMENTS IN THE BIRMINGHAM DISTRICT

Immediately following successful mark-release-recapture experiments in the summer of 1955 in an unpolluted wood in Dorset (recorded later in this paper) we moved camp to the Christopher Cadbury Bird

TABLE 1

Release experiments for B. betularia *(males only)*
Rubery, near Birmingham, 1955

Date	Releases			Total	Catches			Total	Recaptures			Total
	C	T	I		C	T	I		C	T	I	
8.7	54	23	(5)	82	62	7	5	74	...	...	...	...
9.7	...	...	...	...	73	11	1	85	33	11	0	44
10.7	100	41	(4)	145	51	5	2	58	3	2	0	5
11.7	...	...	...	...	50	7	2	59	46	2	(2)	50
12.7	...	...	...	...	89	7	5	101	0	1	0	1
13.7	...	...	...	...	25	4	0	29	...	...	...	...
14.7	...	...	...	...	53	2	1	56	...	...	...	...
15.7	...	...	...	...	20	2	2	24	...	...	...	...
16.7	...	...	...	...	13	2	0	15	...	...	...	...
17.7	...	...	...	...	20	2	0	22	...	...	...	...
18.7	...	...	...	...	15	2	2	19	...	...	...	...
19.7	...	...	...	...	5	1	0	6	...	...	...	...
20.7	...	...	...	...	10	1	0	11	...	...	...	...
Totals	154	64	(9)	227	486	53	20	559	82	16	2	100

	1955			Total	1953			Total
	C	T	I		C	T	I	
Wild Birmingham population per cent. phenotype (fig. 11)	*86·94*	*9·48*	*3·58*	559	*85·03*	*10·14*	*4·83*	621
Per cent. return of releases = recaptures	*53·25*	*25*	*(22·2)*	100	*27·5*	*13·0*	*17·4*	149

The letters C, T, and I stand for *carbonaria*, *typical* and *insularia* respectively throughout this paper

TABLE 1A

Birmingham recaptures, 1953 (See Appendix)

Date	Observed			Total	Expected		
	C	T	I		C	T	I
25.6	5	1	2	8	2·50	3·00	2·50
26.6	0	0	0	0	0	0	0
27.6	0	1	2	3	1·68	0·56	0·76
28.6	9	4	2	15	8·81	5·00	1·19
29.6	0	0	0	0	0	0	0
30.6	17	2	1	20	13·89	5·10	1·57
1.7	41	6	0	47	37.11	8·66	1·24
2.7	30	2	1	33	24·92	7·07	1 01
3.7	26	2	0	28	22·94	5·06	0
4.7	12	0	0	12	10·18	1·52	0·30
Totals	140	18	8	166	121·46	35·97	8·57

Reserve near Birmingham, which I had chosen in 1953 as being likely to offer the optimum conditions for my requirements. I had three objects in view in this undertaking. Firstly to repeat a small mark-release experiment similar to that of 1953. Secondly, to give Dr Tinbergen an opportunity of filming the experiments so as to make

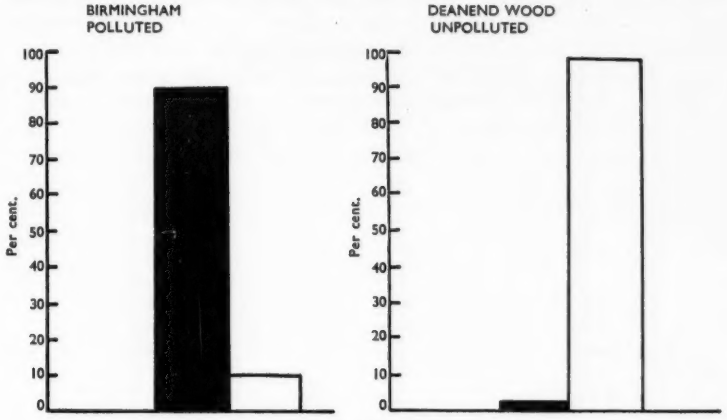


FIG. 1.—Local Population Frequencies.

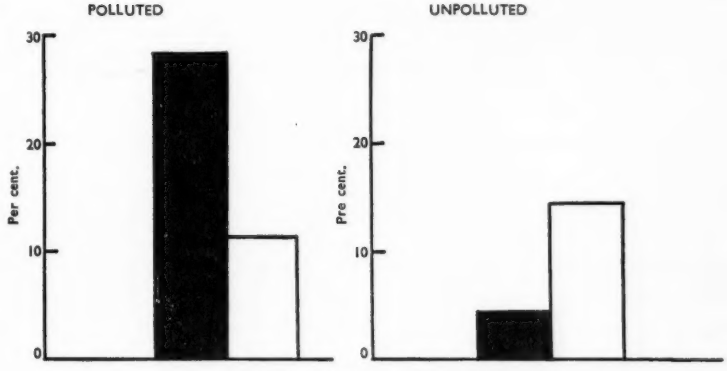


FIG. 1*a*.—Recapture Frequencies.

a visual record of them in the same way that he had done previously in Dorset, and thirdly to check the local phenotype frequencies, after having released 137 male and 34 female *typical betularia* in 1953.

(a) *Mark-release-recapture results*. Altogether a total of 227 *betularia* were released over two periods, 154 *carbonaria*, 64 *typical* (and 9 *insularia*), see table 1. Of these, I recaptured a total of 100, 82 being *carbonaria*, 16 *typical* (and 2 *insularia*), see tables 1 and 1A. This represents a

return of 52·25 per cent. of the *carbonaria* release and 25 per cent. of the *typical* (*insularia* 22·2). Once again we recaptured twice as many of the black form as of the light, but this differed from the 1953 releases which were spread over 11 days, when we recaught 27·5 per cent. of *carbonaria* and 13 per cent. of *typical*. Whether the present increased return rate was due to the greater efficiency of our recapture

TABLE 2

Observation on predation of B. betularia, *by Redstarts for 2 days only (by Dr N. Tinbergen from his hide), Birmingham, 1955*

	Typical	*Carbonaria*	Total
19.7 a.m.	12	3	15
20.7 a.m.	14	3	17
p.m.	17	9	26
Total . .	43	15	58

N.B.—Replenished to equality as soon as all three of either phenotype had been taken.

methods, or to decreased predation, diminished migration or a small natural population, I am unable to say. But it is important to note that the *relative* return figures were of the same order as in my previous series of experiments.

(b) Dr Tinbergen succeeded in filming Redstarts (*Phœnicurus phœnicurus* L.) taking and eating our releases (plate II, fig. 5), and I am able to give in table 2 his records of the predation which took

TABLE 3

Comparative methods of collecting at M.V. light and assembling traps for 6 nights only. Birmingham, 1955

	8th July			9th July			10th July			11th July			12th July			13th July			Totals		
Phenotypes	C	T	I	C	T	I	C	T	I	C	T	I	C	T	I	C	T	I	C	T	I
M.V. light traps	32	6	2	9	5	0	23	4	2	19	1	0	72	7	4	12	2	0	167	25	8
Assembling traps	30	1	3	97	17	1	31	3	0	77	8	4	17	1	1	3	0	2	255	30	10

place on two days whilst he kept observation from a hide. In each case the two forms were released in equality and subsequently replenished at intervals *after all of one form had been taken*. In this way, he recorded that 43 of the pale *typical* form were eaten to 15 of the black form *carbonaria* and, on the majority of occasions, two or more *typicals* were eaten before a *carbonaria* was discovered. Once again

we were able to show that the presence of a conspicuous light coloured *typical* immediately put the better hidden *carbonaria* at a disadvantage which they would not otherwise have incurred if they had been released on their own.

(c) A total of 559 wild *betularia* were caught over a period of 13 nights, *carbonaria* 486, *typical* 53 and *insularia* 20, giving the percentages respectively of 86·94, 9·48 and 3·58 (see table 1). This compares with 621 *betularia* taken over 11 nights in 1953 with *carbonaria* 85·03 per cent., *typical* 10·14 per cent. and *insularia* 4·83 per cent. Furthermore, table 3 demonstrates that for the 6 nights on which light and assembling traps were both in operation the total catch (= wild population+releases) showed a similar proportion of each phenotype as coming to both methods of collecting. It again compares with the high degree of consistency with my 1953 figures, see table 3A.

TABLE 3A

Comparison of the proportion (per cent.) of phenotypes which came to M.V. light and to assembling

Year	Birmingham 1953 (for 10 nights)				Birmingham 1955 (for 6 nights)			
Phenotypes	C	T	I	Total	C	T	I	Total
M.V. light—								
Totals	263	47	24	**433**	167	25	8	**200**
Percentage	*83·60*	*10·85*	*5·55*	*100*	*86·44*	*10·17*	*3·39*	*100*
Assembling—								
Totals	281	34	13	**328**	255	30	10	**295**
Percentage	*85·68*	*10·36*	*3·96*	*100*	*83·5*	*12·5*	*4*	*100*

This small repeat experiment fully corroborated the findings of the previous one, namely that the pale *typical* form, as found in unpolluted countryside, is at a cryptic disadvantage in an industrial area. The fact that birds eliminate these selectively, thereby affecting the evolution of the Peppered Moth, was again recorded.

3. SELECTION EXPERIMENTS IN UNPOLLUTED COUNTRYSIDE

It can be presumed that melanic mutations take place at intervals throughout the range of *Biston betularia*. To test their selective advantages or disadvantages by mark-release-recapture experiments, it would be advantageous, therefore, to choose a location where the melanic *carbonaria* was only maintained by recurrent mutation. A wood having such specifications and, at the same time, suitable in other requirements such as offering isolation and free access by rides was not easy to find. Moreover, amenities for housing the very large

number of pupæ necessary to ensure specimens for release had to be available on the site. A large number of woods in Devon and Cornwall, where only the *typical* light coloured form of *Biston betularia* occurs, were investigated, but were found to be unsuitable for one reason or another. Eventually a peninsula of extremely heavily lichened woodland was found in Deanend Wood, Dorset (fig. 2), and in 1954 I was able to visit it for a single night with a view to collecting a random sample of *betularia* Twenty specimens were taken assembling to virgin females, of which 19 were *typical* and 1 *insularia*. There were

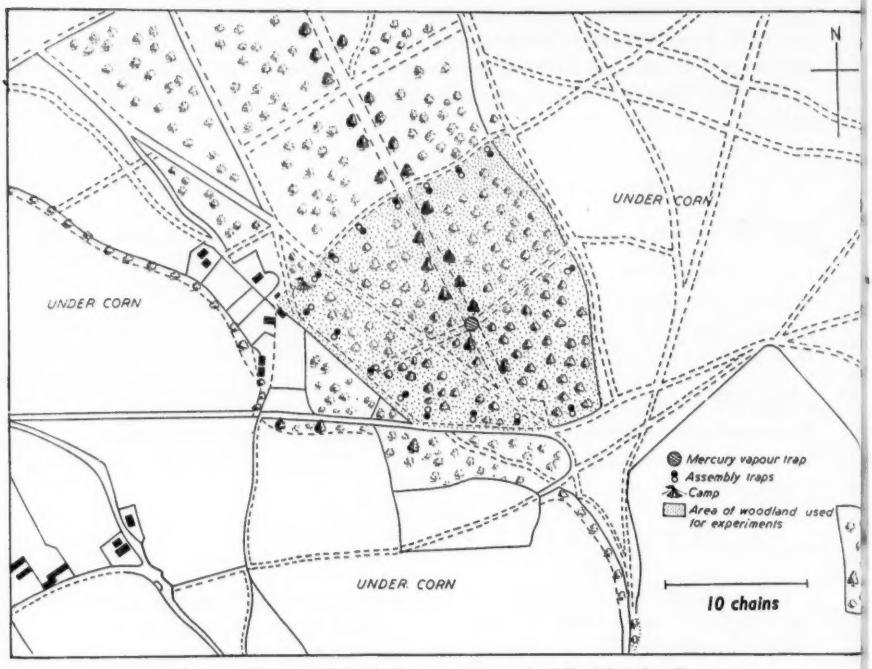


FIG. 2.—Deanend Wood, Dorset. Drawn by Miss Christine Court.

no *carbonaria*. Furthermore, leaf washings from locally collected samples showed a small degree of pollution only. Accordingly, in 1955, in mid-June, I set up camp in these woods. Suitable sheds were found to house the three thousand *betularia* pupæ. An electric generator with mercury vapour traps was installed in the centre ride and the periphery of the wood was lined with cages destined to contain virgin females, so that at night the whole release area would be subject to a concentration of female assembling scent and this, no doubt, succeeded in holding a proportion of our male releases within the area of woodland.

The experiments were designed so that each aspect of the work could be compared with the previous Birmingham results, and the methods employed came under the following headings :

(1) Scoring values as gauged by human standards.
(2) Direct observation as to what happened to the individuals so scored.
(3) Recapture figures which provided data over longer periods.

On every occasion, recapture results were assessed on male Peppered Moths only, because of the impossibility of recapturing females. However, these were used as releases for continuous observation from hides where, at the same time as a film record which was made, the order of predation of each insect was noted.

(i) *Scoring values for crypsis*

The technique of scoring previously used was to assess, in the first place at a distance of two yards, whether a released moth was (*a*) conspicuous, or (*b*) inconspicuous. Subsequently, three categories were allowed for each, so that the score for (*a*) would be either −1, −2, or −3, and for (*b*) +1, +2, or +3, depending on the distance at which the insect faded into its background. As pointed out in the previous paper, this method of scoring worked satisfactorily in the Birmingham experiments. It came as a surprise then to find that, of 120 *typicals* released in Deanend Wood during the first few days, all were classed as inconspicuous, with 85 per cent. scoring +3. Conversely, all 75 *carbonaria* scored by us appeared conspicuous and 80 per cent. of these scored −3. In view of the random sample of the release points selected, it appeared unnecessary to continue the arduous procedure of scoring the cryptic value of each individual release, and it was accepted that in these woods, to the human eye, the majority of the light *typical* form were extremely well hidden, and the *carbonaria* were nearly always conspicuous (plate I, fig. 1).

(ii) *Direct observation*

(a) *A record of the number of individuals present or absent by late afternoon.* Early on in the experiments an apparent deficiency of the light *typical* form was recorded when the afternoon check on releases took place. There were also many *carbonaria* missing. In the present work, in regard to the other melanic *insularia*, the numbers used, though quoted, were so small that they have no significance. It became increasingly obvious that one was passing over the *typical* form on the lichened tree trunks, and they are practically impossible to see. To test this, I did a check immediately following the morning's release, in an area where my continual presence prevented predation. All the *carbonaria* were present, but over 30 per cent. of the *typicals* were unaccounted for. The cryptic efficiency of the *typical* on a lichened

background is, in fact, greater than that of *carbonaria* on the blackened Birmingham tree trunks. For this reason, this type of recording was discontinued.

(b) *Observation from hides.* In the course of filming, Dr Niko Tinbergen, who had to spend the greater part of each day in a hide, recorded the order in which predation occurred. These figures are added to my own observations. It must be emphasised that these records were of concentrations of female *betularia* necessary for photography, but that they played no part in my release-recapture figures. On each occasion, an equal number of black and white forms were used at the commencement, but it was found impracticable to replace each phenotype after it had been taken. Their numbers were

TABLE 4

Direct observation on predation by five species of birds. Deanend Wood, Dorset, 1955

	Observer	*Carbonaria*	*Typical*	
Spotted Flycatcher (*Muscicapa striata* L.)	N. T.	46	8	
	H. B. D. K.	35	1	
Nuthatch (*Sitta Europaea* L.)	N. T.	22	8	
	H. B. D. K.	9	0 (first day)	
	H. B. D. K.	9	3 (second day)	
Yellow Hammer (*Emberiza citrinella* L.)	N. T.	8	0	
	H. B. D. K.	12	0	
Robin (*Erithacus rubecula* L.)	N. T.	12	2	
Thrush (*Turdus ericetocum* L.)	N. T.	11	4	
Total predation observed (for days when records were kept)	...	164	26	Total 190

Note.—On all occasions these observations commenced with equal numbers of both phenotypes. We replaced them when all of one phenotype had been taken.

replenished after the last of one phenotype had been eaten, thus preventing simple statistical analysis. By doing this, it will be appreciated that the bias was, thereafter, in favour of that phenotype which had been eliminated previously, and it was found that on the majority of occasions the more conspicuous of the two forms were all taken before any of the others. This behaviour was common to five species of birds to a greater or lesser degree, as will be seen from the figures in table 4 (plate I, fig. 3, and plate II, figs. 1, 2, 3 and 4).

(iii) *Recapture results*

Releases were undertaken on fourteen occasions in all, making a total of 969 individuals, 473 being *carbonaria* and 496 *typical*. I

recaptured 30 *carbonaria* and 62 *typical*. For all types of release we got back 12·5 per cent. of the *typical* form, but only 6·3 per cent. of the *carbonaria*. For various reasons about to be discussed, the releases on three days should be excluded from this total as they are unduly biased in one way or another. This gives a figure of 799 releases

TABLE 5

Release experiment figures for Biston betularia (*males only*).
Deanend, Dorset, 1955

Date (1953)	Releases			Total	Catches			Total	Recaptures			Total
	C	T	I		C	T	I		C	T	I	
(13.6)	(37)	(38)	(9)	(84)	...	...	...	...	...	...	...	...
14.6	8	17	2	27	4	17	2	23	(4)	(1)	(1)	(6)
(15.6)	(8)	(25)	(1)	(34)	2	27	5	34	2	9	(3)	14
(16.6)	(22)	(40)	(0)	(62)	1	34	2	37	(0)	(0)	(0)	(0)
17.6	...	...	...	...	7	58	3	68	(7)	(7)	(0)	(14)
18.6	42	65	(3)	110	0	30	1	31	0	1	0	1
19.6	39	72	0	111	2	26	1	29	2	6	0	8
20.6	24	57	0	81	1	44	2	47	1	3	0	4
21.6	42	29	0	71	1	13	2	16	1	4	0	5
22.6	...	...	...	...	5	13	1	19	5	4	0	9
23.6	...	...	...	...	...	No releases		...	...	No captures		...
24.6	...	...	...	...	...	No releases		...	...	No captures		...
25.6	82	43	0	125	1	11	0	12	0	0	0	0
26.6	...	...	...	...	3	8	1	12	2	2	0	4
27.6	51	28	0	79	0	8	0	8	0	0	0	0
28.6	22	22	0	44	1	20	0	21	1	5	0	6
29.6	17	18	0	35	0	14	0	14	0	5	0	5
30.6	24	11	0	35	4	11	1	16	3	6	0	9
1.7	...	...	...	...	2	9	0	11	2	2	0	4
2.7	...	...	...	...	0	7	0	7	0	0	0	0
3·7	...	...	...	...	...	...	...	...	...	...	...	...
4·7	55	31	0	86	...	...	...	...	...	...	...	...
5·7	...	...	...	...	0	9	0	9	0	7	0	7
Totals	**473**	**496**	**15**	**984**	**34**	**359**	**21**	**414**	**30**	**62**	**4**	**96**

	C	T	I	Total
Wild Deanend population .	(4)	297	17	**318**
Per cent. phenotype (fig. 11) .	(Possible escapes)	*94·60*	*5·4*	
Release after 1 day of self-determination	2	4	(1)	
Per cent. phenotype . .	...	...	...	
Per cent. return of releases .	*6·34*	*12·50*	*(26·67)*	

for the 11 correct days : 406 being *carbonaria* and 393 *typical*. I got back 19 *carbonaria* and 54 *typical*, being 4·68 and 13·74 per cent. respectively (see tables 5 and 5A).

4. DISCUSSION AND RELEASE PROBLEMS

Various types of release techniques were undertaken with the object of finding the best way of subjecting as many individuals as

possible, under natural conditions, to maximum predation for the longest time. I propose, therefore, to discuss these in some detail, and to give reasons for excluding three sets of figures from the table due to the unsatisfactory nature of the releases.

I used 84 *betularia* in the first experiment (13/6/55). These were released on only twenty trees, the reason for this small number of release points being that I had been unable to locate and prepare the other trees in the area. The result was that I produced a high concentration of moths on comparatively few tree trunks (an average of 4 per tree), nor were the two forms on every occasion released in equality per tree. The late afternoon check showed that in nearly

TABLE 5A

Deanend recaptures, 1955

Date	Observed			Total	Expected		
	C	T	I		C	T	I
(13.6)	(6)	(3)	(3)	(12)	(5·29)	(5·43)	(1·29)
14.6	1	7	2	10	2·96	6·30	0·74
(15.6)	(0)	(1)	(0)	(1)	(0·24)	(0·74)	(0·03)
(16.6)	(8)	(8)	(0)	(16)	(5·68)	(10·32)	(0)
17.6	...	...	...	...	...	...	...
18.6	2	8	0	10	3·81	5·91	0·27
19.6	1	2	0	3	1·05	1·95	0
20·6	2	4	0	6	1·78	4·22	0
21.6	4	4	0	8	4·73	3·27	0
22.6	...	...	...	...	...	...	...
23.6	...	...	...	...	...	...	...
24.6	...	...	...	...	...	...	...
25.6	2	2	0	4	2·62	1·38	0
26.6	...	...	...	...	...	...	...
27.6	1	5	0	6	3·87	2·13	0
28.6	0	7	0	7	3·50	3·50	0
29.6	3	8	0	11	5·34	5·66	0
30.6	2	1	0	3	2·06	0·94	0
4·7	0	7	0	7	4·47	2·52	0
Totals	32	67	5	104	47·40	54·27	2·33

every case the moths were either all present or all absent per tree. Subsequently, by direct observation, Dr Tinbergen and I found that a concentration of releases increased the predation risk for all present, even though the birds took the more conspicuous ones first. It will be noted that on this occasion we recaptured 4 *carbonaria* and 1 *typical* (expected—near equality). It is possible that, apart from a faulty release technique, a further point may have played a part in the production of these figures. The birds in this wood were unlikely to have had any previous experience of the Black Peppered Moths, and it is conceivable therefore that at first some of them did not recognise them as an article of diet. Dr Tinbergen did, in fact, record a similar incident in one of the Birmingham 1955 experiments. A Wren

(*Troglodytes troglodytes* Linn.) flew on to a tree trunk on which was released a very conspicuous *typical* (Birmingham frequency 10 per cent.) and after scrutinising it closely it flew away without attacking it. It is, however, possible that the noise of the ciné camera disturbed it at this point.

The next release (14/6/55), which was considered satisfactory, was conducted with a greatly lowered concentration. Twenty-seven *betularia* were, in fact, put on to 23 trees.

The release on the 15th was designed to test whether the presence of a *carbonaria* on the same tree trunk as a *typical* lowered the latter's expectation of survival. Apart from the 8 male *carbonaria* released, females had, on this occasion, to be used also (because of a temporary lack of *carbonaria* males), so that each of the male *typicals* had a black moth on the same tree trunk. Two trees, however, were used as controls, and on these two *typicals* were released on each, with no accompanying melanics. The late afternoon examination (6 p.m.) gave the surprising result that no *betularia* of any form were to be found on any of the release trees, with the exception that one of the control trees (without a *carbonaria* being present) had two *typicals* on it. The following night, out of 37 *betularia* caught at light and assembling traps, none were from the releases of the 15th. The next night, however, I recaught one *typical* (out of 68) from this release. This would seem to provide additional evidence to the statement I made in my original paper (p. 336), " From this it would appear that, when a conspicuous insect had been found, it at once put other insects in the immediate vicinity at a disadvantage because of the bird's active seachings." For these reasons I feel that the release conducted on the 15th should not be included.

The following day (16/6/55) a release was undertaken between 5 and 6 p.m., long after the maximum predation time of birds. The object was to test whether, having excluded predation, the design of experiment favoured unduly one phenotype more than another. Twenty-two *carbonaria* and 40 *typical* were released. Of these, 7 *carbonaria* and 6 *typical* were retrapped the same night (expected—4·61 to 8·39). It will be noted that this represents the return of nearly a quarter of our releases on the 16th. I am unable to account for the increased proportion of *carbonaria* recaptures over *typical*, which occurred this night, but in view of the absence of predation it suggests that the recapture arrangements did not unduly favour the return of the *typicals* more than the *carbonaria*. We have, however, already shown that in the Birmingham experiments there was no difference in the proportion of the three phenotypes which were collected, firstly, at mercury vapour light traps and, secondly, at assembling traps containing females of all three phenotypes (proportions of *carbonaria*, light and assembling, 85 per cent. to 86 per cent., and *typical* 10·8 to 10·4 per cent.). Furthermore, we are able to report similar findings in this present set of experiments (see table 6). In view of these facts,

and that this release was not designed to reflect the degree of selective predation, and that little in fact could have taken place, it is better to extract the results, along with those of the other two previously mentioned, from those which are about to be considered.

It can be seen from this that the act of predation is no simple response to a single stimulus. It involves, apart from insect cryptic efficiency, such other considerations as insect density, bird conditioning, and searching intensity per trunk, stimulated by an immediate previous experience of finding a conspicuous insect. All other releases were in fact conducted in a uniform manner (with the exception of one other satisfactory method used). In these, on every occasion, there was one phenotype released per tree, and the experiment was conducted over a larger area of woodland. Furthermore, as in the Birmingham experiments, other species of moths which were inhabiting the wood were released within the area at the same time, to minimise

TABLE 6

Comparative methods of collecting at M.V. light and assembling traps. Deanend, 1955

Phenotypes	*Carbonaria*	*Typical*	*Insularia*	Total	
M.V. light—					
Totals	14	166	12	192	414
Percentage	*7·29*	*86·77*	*5·94*	*100*	
Assembling—					
Totals	20	193	9	222	
Percentage	*9·01*	*86·94*	*4·05*	*100*	

the effect of conditioning. This, of course, involves a great deal of extra work, but it is necessary. One further point was noted, that the same trees must not be used as release points on each day, as the birds became conditioned to them.

The " other method " of release referred to was used successfully on 18th June. This took place just before sunrise, between 4 and 4.30 a.m. Forty-two *carbonaria*, 65 *typical* (and 3 *insularia*) were allowed to fly out of their separate boxes which had been previously warmed on the engine of my car. The majority flew and took up positions on the boughs and trunks of nearby trees. I, therefore, used many release points within the area. This method was not repeated because it is necessary to get each insect airborne over a very short period of time, which was difficult due to the coldness of the morning, so that by the time the last few flew, birds were active and, in fact, a Spotted Flycatcher (*Muscicapa striata* L.) chased and caught two *typical betularia*. A too early release, on the other hand, would involve a number of the moths coming to the various traps in action from the previous night. With the exception of the two *typicals* taken by birds,

it appeared that this release was satisfactory. One *carbonaria* and 6 *typical* were subsequently recovered.

We have produced evidence that, in undertaking any release experiments involving the use of cryptic insects which normally pass the day concealed on their appropriate backgrounds, due regard must be taken of such factors which affect predation as density, proximity of an individual which has been scored conspicuous, bird conditioning for recognition of a particular species, or for the place of release. To avoid all these complications, individual releases, undertaken over a large area containing many trees, are essential.

5. CONCLUSIONS

We are now in a position to review firstly, two separate series of release experiments conducted in an industrial district : secondly, to compare figures obtained from these with the data from a similar release undertaken in an unpolluted and heavily lichened wood in Dorset.

In regard to the Birmingham experiments : in the second of the two series which were carried out, scoring for crypsis was not repeated as it was unnecessary ; neither the trees nor the phenotypes having altered since 1953. Direct observation on bird predation in each case showed that the *typical* light form was eaten more frequently than *carbonaria*, and the deficiency of this light form in our recapture figures can be attributed in each case to selective elimination by birds, as such other considerations as sampling errors, a different life span or migration rate for each phenotype, can be ruled out of the repeat experiment for the same reasons as given in 1953.

On comparing the Birmingham figures for crypsis with those of Deanend Wood, Dorset, we found a complete reversal. Over 97 per cent. of the *carbonaria* in the former location were scored inconspicuous, whereas 89 per cent. of the *typicals* were adjudged conspicuous. In Deanend Wood, of the *carbonaria*, all were scored conspicuous, whilst of the *typicals* all were inconspicuous. Furthermore, of these, 85 per cent. were given the highest mark for cryptic efficiency.

Consequently the observed bird predation on both occasions in Birmingham showed elimination in favour of *carbonaria*. In 1953, a total of 18 *betularia* were kept under continuous observation, half being *carbonaria* and half *typical*. All the nine *typicals* were observed to be eaten by Robins and Hedge Sparrows, but only 3 *carbonaria*, the remaining 6 were never discovered and, in fact, survived the day up to 7 p.m. In the recent work, Dr Tinbergen recorded that a pair of Redstarts and their young took 43 *typicals* to 15 *carbonaria* during experimental releases in which both black and light forms were used in equality (table 2).

On the other hand, in Deanend Wood, Dorset, the reverse was observed : 5 species of birds took 190 *betularia* whilst being watched (table 4). Of these, 164 were *carbonaria* and 26 *typical*. It must be

accepted, therefore, that the *carbonaria* has an approximate 6 : 1 advantage in Birmingham, and the reverse was true for Deanend. In regard to the recaptures, on each occasion in Birmingham I got back twice as many *carbonaria* as *typicals*, but in Deanend I recaptured three times as many *typical* as the melanic (text fig. 1*a*). Furthermore, if the collecting techniques employed were comparable in each case, it would appear that the predation intensity was greater at Deanend. Fewer individuals of each phenotype survived. This is also reflected in the nightly recapture totals (assuming, once again, that our collecting efficiency was comparable). In the 1953 large-scale Birmingham experiments, the average return within 24 hours was 19 *betularia* per 100 releases. For Deanend, it was 9 per 100. It is, however, the selective predation and not the total predation with which we are concerned in these present investigations.

6. SUMMARY OF ALL THREE EXPERIMENTS

1. The Peppered Moth, *Biston betularia*, is one of about seventy species of moth which are at present in the process of changing their populations from light to dark individuals. The common Industrial Melanic = form *carbonaria* is black, and another = form *insularia*, which is not an allelomorph, varies from light to heavily speckled specimens (plate II, fig. 6).

2. This paper records two complementary series of experiments, involving the release of nearly 2000 marked moths, to test the relative camouflage or cryptic advantages of the normal light-coloured *betularia* and its melanic form *carbonaria*. This was carried out in the first place near the industrial area of Birmingham where the *carbonaria* form represents about 87 per cent. of the population. Secondly, a similar experiment was undertaken in a heavily lichened and pollution-free wood in rural Dorset, where *carbonaria* does not normally occur or, if it does, at a very low frequency. (The other melanic, *insularia*, though mentioned, was used in too small numbers to have significance.)

3. The first series was a repeat of similar work I carried out in 1953, and fully corroborates the conclusions previously published (Kettlewell, 1955).

4. On each occasion, the more conspicuous of the two forms was deficient in numbers amongst the recaptures ; *typical betularia* in Birmingham and *carbonaria* at Deanend Wood, Dorset.

5. (i) Scoring for conspicuousness as recognised by Man, and (ii) direct observation of the released insects showed that birds were responsible for their elimination, both in polluted and unpolluted countryside, and this took place selectively in each case, and in an order which varied according to the camouflage efficiency of each phenotype in relation to its background.

6. In unpolluted and heavily lichened countryside melanic forms are maintained only by recurrent mutation, and are rapidly eliminated because of their conspicuousness.

Plate I

FIG. 1.—*Typical betularia* (left) and its melanic *carbonaria* (right) at rest on lichened tree trunk, Deanend Wood, Dorset.

FIG. 2.—*Typical betularia* and its melanic *carbonaria* at rest on lichen-free tree trunk near Birmingham.

FIG. 3.—Song Thrush, *Turdus ericetorum* L., examining tree trunks from the ground with a *carbonaria* in its beak.

Plate II

FIG. 1.—Nuthatch, *Sitta europæa* L., in the act of taking *typical betularia* from lichened tree trunk, Deanend Wood, Dorset. This species took 40 *carbonaria* to 11 *typical* while under observation.

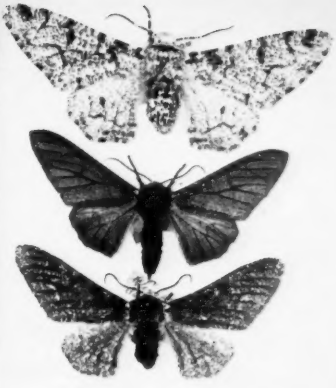
FIG. 2.—Spotted Flycatcher, *Muscicapa striata* L., about to take *carbonaria* from oak trunk, Deanend Wood, Dorset. This species was seen to take 81 *carbonaria* to 9 *typical*.

FIG. 3.—Robin, *Erithacus rubecula* L., with *carbonaria* in its beak taken from lichened tree trunk, Deanend Wood, Dorset. There were 3 *typicals* on this trunk at the moment of this photograph being taken. This species took 12 *carbonaria* to 2 *typical* whilst being watched.

FIG. 4.—Yellowhammer, *Emberiza citrinella* L., searching tree trunk, Deanend Wood, Dorset. A pair took altogether 20 *carbonaria* and on no occasion whilst under observation did they discover the *typical* form which, on every occasion, was offered in equal numbers to the black.

FIG. 5.—Male Redstart, *Phœnicurus phœnicurus* L., with *typical betularia* in its beak in a wood near Birmingham. This species took 43 *typical* to 15 *carbonaria* whilst under observation.

FIG. 6.—*Typical Biston betularia* L., its melanic *carbonaria* Jordan, and f. *insularia* Th-Mieg, another melanic.



7. In industrial areas this limitation no longer exists, in fact it is the pale-coloured *typical* which is now eliminated. This applies also to areas far to the east of them, because of the prevailing westerly wind, where large areas of England, subject to pollution " fall-out ", are deficient in lichens.

8. Predation alone is responsible for the fact that in the Birmingham district I got back only 50 per cent. of the *typicals* that were expected from the proportions of the two forms released. In contrast, at Deanend, it was the *carbonaria* form which was deficient. In fact I recaptured only 67 per cent. of the number expected.

9. The difference in cryptic coloration alone could be responsible for the rapid spread of the Industrial Melanics. There are also, however, other character and behaviour differences between them and their *typical* forms. These are at present the subject of investigation.

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7. REFERENCE

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APPENDIX

Table 1A (p. 288) reflects a different method of analysing the 1953 recaptures, to that shown in the previous paper. The original recapture figures (Kettlewell, 1955, Table 5) record the number of marked individuals which returned each night. The present table takes into account the number of days each individual had been in the wild. Thus a recaptured insect showing three marks is entered under each of the three releasing days separately.

YIELD IMPROVEMENT FROM A SELECTION INDEX TECHNIQUE WITH COTTON

H. L. MANNING

Empire Cotton Growing Corporation, Uganda and the Institute of Statistics, North Carolina State College, Raleigh

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1. INTRODUCTION

PLANT breeders can usually express their aims in simple terms of yield or quality, which affect the economic net worth of their crop. To attain these aims, however, requires measures of the traits involved and a system of weighting according to their phenotypic importance and their genetic variability and inter-relations. In the cotton breeding project here reported, quality was at a satisfactory level and improvement of yield was the main object. It was therefore easy to measure the important characters. There are three components of yield, namely, bolls per plant, seeds per boll and lint per seed. In breeding projects in which quality or disease resistance are of importance, objectivity, though difficult to achieve, should not be impossible.

Hazel and Lush (1942) have indicated that, when several traits affect the net worth of an organism, it is necessary to weight variations in those characteristics in the proper ratio if maximum progress from selection is to be obtained. A method for estimating optimum relative weights has been given by Fairfield Smith (1936), Hazel (1943) and Lerner *et al.* (1947) for various selection projects. More recently, Robinson *et al.* (1951) have given procedures for estimating genotypic and phenotypic covariances required for the construction of a selection index.

The primary purpose of this paper is to report on the results achieved by using a selection index technique with a continuously self-fertilised variety of cotton. The unexpected behaviour of an open-pollinated stock, maintained by a form of modal selection to furnish check yield standards, is also of some interest. Finally, there is some discussion relating to the persistence of genetic variability after seven generations of self-fertilisation.

2. MATERIAL AND PROCEDURE

The BP52 variety of cotton traces to a single plant selection from the Nyasaland Upland stock. It was maintained in open-pollinated bulks for ten years during which some selection was exercised for quality and yield. At the end of 1944, about 800 plants were selected in a field, largely on the basis of sympodial habit and productivity, but also for leaf hairiness. Part of the seeds of each of the plants exhibiting the greatest leaf hairiness, was set aside in storage. Of the remainder, plants with seed cotton yield above the mean were bulked to form a new strain, then designated BP52B. An open-pollinated bulk of the descendants of this strain is now used as the check variety and has been designated K/51.

A mixture of BP52 material was made up from some of the stored selections, single plant produce from outlying stations in Uganda, some open-pollinated single plant selections made in commercial fields during the 1945-46 season, and samples from the bulked seed of the commercial variety and K/51. This material was planted in 1946 in an area of about 1½ acres, which would provide a population of about ten thousand plants. Four hundred and twenty plants were selected from this area, mainly on the basis of sympodial habit and of their apparent productivity. Reaping was confined to a single period when 35 to 50 per cent. of the plant produce was available. Twenty-three plants were outstanding for lint yield but with lint indices of not less than 3 centigrams of lint per seed. These gave 23 progenies which were grown in non-replicated rows in the following season. From these all subsequent progeny row material has descended. The remaining 397 plants were used to constitute a " modal " bulk check standard which will be described later.

The replicated progeny row system with cotton was first introduced by Hutchinson and Panse (1937) and has since been used extensively on stations of the Empire Cotton Growing Corporation. In this project, self-fertilised seed from each selected plant was divided into two parts. The first was grown in a replicated experiment, with a suitable number of replications, each plot containing 10 or 12 plants at a spacing of 3 feet by 2 feet. Though from 3 to 5 seeds were sown in each hole, the plants were singled, after full emergence, some 15 to 20 days after planting. With 5 replications, progeny means would thus be based on from 50 to 60 plants. The remaining sister seed, of the original selected plant, was grown nearby in a single non-replicated row of from 30 to 60 plants all of which were self-fertilised. Progeny performance in the replicated trial provided the basis for selection. Representative plants were then picked in the non-replicated portions of the selected progenies, but it is not suggested that any significant advance was made by within progeny selection. In each of the past seven seasons from 30 to 81 progenies have been grown in either Randomised Complete Block or Lattice Square arrangements. The gain in efficiency from the latter has been so low that the simpler lay-out is now in general use.

The self-fertilised single plant selection (from the non-replicated row) of any one season becomes the parent of a progeny of from 70 to 120 plants. In turn the progeny of two or more Sib selections are said to belong to the same *strain*. Several strains are said to constitute a *line* when descended from a common great-grand-parental plant. In this nomenclature, therefore, there may be lines, strains and progenies, originally from a single plant. Namulonge BP52 stocks are designated by the letter C, followed by the year in which the particular material was grown as a progeny, and the serial number of the progeny in that year. Thus C50(20) was the twentieth progeny of the 1950 series. For details see Fig. 1.

Total lint yield was measured in the replicated trials (in contrast to the partial yield in modal bulks). This may require from 6 to 10 reapings. A bolling index, though estimated separately for each plant, is given on a single plot basis, and is derived from counts of the mature bolls about the time of the first natural boll-split. A second trait, seeds per boll, is estimated from a 15 boll sample from each plot, obtained from the second and third reapings. A third trait, lint per seed (also known as lint index), is estimated from the entire produce, number of seeds being estimated from seed weight per seed in a 100 seed sample.

Yield advance actually achieved, is tested in large scale trials with plot sizes of about 1/50 acre. In one series of trials, bulked open-pollinated progenies are compared with a modal bulk check. The second series consists of thirteen 5×5 Latin squares at sites chosen to represent the soil types and climatic conditions experienced by the commercial crop. In consequence, strains are tested under a very much wider range of environmental conditions than the single experiment station where the programme was conducted.

3. CONSTRUCTION OF SELECTION INDEX

(i) *Characters measured*

The objective of selection will always be to effect population changes in one or more traits which relate to the economic value of the organism. Whatever these traits may be some function of them will represent economic net worth, which may be symbolised as W. Since phenotype is conditioned by both genotype and environment, W may be represented as the sum

$$W = G_w + E_w$$

where G_w is average genotypic net worth and E_w is a deviation from the average associated with the particular environment in which phenotype is observed. G_w is the quantity to be improved by selection. The most efficient basis for selection, *i.e.* the optimum selection index, would appear to be one having the largest possible correlation with G_w. An obvious index would be W itself but intuition may often suggest that special attention be directed to characters having unique significance with respect to yield or quality. In fact any index of the form

$$I = b_1X_1 + b_2X_2 + \ldots b_nX_n$$

(where X_1, X_2, ... X_n represent objective measurements or numerical scores on any series of individual traits) may be considered. The magnitude of correlation ρIG_w, between an index and net worth provides a rational criterion for choice among indices; the index for which ρIG_w is maximum being the superior one. Expressed differently, where net worth is affected by two or more individual traits, selection may be employed to alter the genotype in a variety of ways all of which may increase worth, but of these ways, which will differ because of relative emphasis placed on different traits, one will be the best because weighting is optimum.

Fairfield Smith (1936) and Hazel (1943) demonstrated that the optimum weights, b_1, b_2, ... b_n, to be given n characters on which phenotypic data have been taken, are functions of (1) the genetic and phenotypic variances and covariances of the traits, and (2) the relative contributions of the traits to phenotypic net worth, all properly recognised. They showed that the optimum b's are obtained from the following set of equations solved simultaneously.

$$\left.\begin{aligned} b_1t_{11} + b_2t_{12} + \ldots + b_nt_{1n} &= g_{1w} \\ b_2t_{12} + b_2t_{22} + \ldots + b_nt_{2n} &= g_{2w} \\ &\cdot \\ &\cdot \\ &\cdot \\ b_nt_{1n} + b_2t_{2n} + \ldots + b_nt_{nn} &= gn_w \end{aligned}\right\} \quad . \quad . \quad (1)$$

where the t's are either phenotypic variances of progeny means for single traits (t_{11}, t_{22} ... t_{nn}) or the corresponding phenotypic covariances for pairs of traits (t_{12}, t_{13}, t_{23}, etc.); and g_{1w}, g_{2w} ... g_{nw} are genetic

covariances of n single traits with net worth.* It should be emphasised that the parameters are defined with reference to the population of progenies in which selection is actually practised.

Procedures for estimation of the t's and g's and examples of selection indices have been given by Fairfield Smith (1936), Hazel (1943), Lerner *et al.* (1947) and Robinson *et al.* (1951). Details of the procedure followed in the work being reported will now be outlined. The traits used, bolls per plant, seeds per boll, lint per seed and total lint yield per plant, will hereafter be identified by subscripts 1, 2, 3 and w respectively.

In view of the manner in which data on boll number and seeds per boll were obtained, a selection index might have used information on all four variables. However, anticipating that the major portion of the information about yield would be reflected in the remaining

TABLE 1

Analysis of variance for bolls per plant X_1 *for the 1954-55 season*

Variance source	d.f.	Mean square	Expectation of mean square
Replications . . .	3	73·60	...
Lines	8	8·51	...
Strains	4	0·31 = M_1	$\sigma_e^2+r\sigma_p^2+rk_o\sigma_s^2$
Progenies in strains . .	47	2·33 = M_2	$\sigma_e^2+r\sigma_p^2$
Plot error . . .	177	1·86 = M_3	σ_e^2
Total . . .	239	...	...

Where σ_e^2 = plot error variance,
σ_p^2 = variance arising from genotypic differences among progenies of the same strain,
σ_s^2 = variance arising from genotypic differences among strains of the same line,
r = number of replications, and

$k_o = \frac{1}{n-1}\left[\Sigma k_i - \frac{\Sigma k_i^2}{\Sigma k_i}\right]$ where k_i is the number of progenies in the i-th strain, n = number of strains and summation is over strains (Snedecor, 1953).

three characters, only these were used in the index on which selection was actually based.

The variance for the bolls per plant data from the replicated progeny rows was analysed as shown in table 1 for the 1954-55 season. Partition of degrees of freedom was as follows: The total for all progenies would be 59. Partition within these would be 8 d.f. for lines, 4 d.f. for strains and 47 d.f. between progenies in strains.

* Both Fairfield Smith and Hazel indicated that if net worth is a linear function of phenotype in individual traits, *i.e.* if

$$W = a_1X_1+a_2X_2+\dots a_nX_n, \text{ then}$$
$$g_{1w} = a_1g_{11}+a_2g_{12}+\dots a_ng_{1n}$$
$$g_{2w} = a_2g_{12}+a_2g_{22}+\dots a_ng_{2n}, \text{ etc.}$$

Here the a's are constants which represent the relative economic importance of the various individual traits. Note that what is here symbolised as g_{1w}, g_{2w}, etc. is symbolised by Fairfield Smith as A_1, A_2, etc. When W is directly measurable in terms of a single trait, the most direct procedure for estimating the b's is through direct estimates of g_{1w}, g_{2w}, etc. used in the set of equations (1) given in the text.

The estimates of phenotypic variances (t_{11}, t_{22} and t_{33}) substituted in equation (1), were those for progenies within strains. They were computed directly from the "progenies in strains" mean square by division of the latter by the number of replications. Thus for the 1954-55 season

$$t_{11} = 2{\cdot}33/4 = 0{\cdot}582$$

The phenotypic covariances were obtained in a similar manner from analyses of covariance of the same form as the analysis of variance in table 1.

TABLE 2

Estimates of genotypic variances and covariances for 7 seasons

Season	g_{11}	g_{22}	g_{33}	g_{ww}	g_{1w}	g_{2w}	g_{3w}	g_{12}	g_{13}	g_{23}
1948-49	4·619	0·082	0·0278	10·604	6·854	0·382	0·310	−0·058	−0·074	−0·060
1949-50	0	0·436	0·0561	0·871	−0·068	0·040	0·161	−0·088	0·081	−0·051
1950-51	0·273	1·542	0·0552	0·613	0·272	0·712	0·025	0·659	−0·110	−0·101
1951-52	3·606	0·452	0·1054	3·671	5·578	−0·386	0·165	−0·362	−0·240	−0·073
1952-53	0	0·142	0·0250	0	−1·079	−0·164	−0·123	−0·192	−0·078	−0·049
1953-54	0·218	0·536	0·0119	0·397	0·257	0·225	−0·001	0·334	−0·067	−0·116
1954-55	0·118	0·338	0·0139	0·505	0·138	0·515	−0·033	0·149	−0·023	−0·068
Sums	8·834	3·538	0·2953	16·661	9·952	1·324	0·504	0·442	−0·511	−0·518
Means	1·262	0·504	0·0422	2·380	1·422	0·189	0·072	0·063	−0·073	−0·074

TABLE 3

Estimates of phenotypic variances and covariances for 7 seasons

Season	t_{11}	t_{22}	t_{33}	t_{ww}	t_{1w}	t_{2w}	t_{3w}	t_{12}	t_{13}	t_{23}
1948-49	7·298*	0·927	0·0480*	14·618*	9·848	0·046	0·316	−0·121	0·090	−0·087
1949-50	4·590	1·259	0·0753*	4·597	2·642	0·075	0·244	0·124	−0·020	−0·077
1950-51	1·028	2·157*	0·0659*	2·772	1·098	0·915	0·077	0·566	−0·083	−0·120
1951-52	6·717*	1·253†	0·1228*	7·591*	5·470	0·102	0·261	−0·183	−0·183	−0·082
1952-53	0·785	0·689	0·0374*	1·963	0·071	0·077	−0·097	−0·050	−0·078	−0·054
1953-54	1·801	0·903*	0·0322†	2·489	1·691	0·274	0·043	−0·176	−0·070	−0·007
1954-55	0·582	1·315	0·0404†	1·535†	0·650	0·555	−0·009	0·004	−0·015	−0·077
Sums	22·801	8·503	0·4220	35·565	21·470	2·044	0·925	0·164	−0·359	−0·504
Means	3·257	1·215	0·0602	5·081	3·067	0·292	0·132	0·023	−0·051	−0·072

Progenies in strains $p = 0{\cdot}01$* ; $p = 0{\cdot}05$†

σ_p^2 was estimated by equating the "progenies in strains" and "plot error" mean squares to their expectations, with the result that $(M_2 - M_3)/r$ provides estimates of the desired parameters. Thus again for the 1954-55 season, the estimate of

$$\sigma_p^2 = (2{\cdot}33 - 1{\cdot}86)/4 = 0{\cdot}118.$$

Corresponding estimates of covariances, due to genotypic differences among progenies of the same strain, were obtained in a similar manner from analyses of covariance. Estimates of σ_p^2 for the three

traits and worth itself and the corresponding covariances are listed in table 2 for all seasons. These are designated $\hat{g}_{11}$, $\hat{g}_{22}$, etc. for variances and $\hat{g}_{12}$, $\hat{g}_{13}$, $\hat{g}_{1w}$ for covariances in as much as they were estimates employed in computation of the selection indices.

Table 3 represents estimates of phenotypic variances and covariances.

(ii) *Computation of the* b *coefficients*

Substituting the estimates of $\hat{g}_{1w}$, $\hat{g}_{2w}$ and $\hat{g}_{3w}$, and the t's from tables 2 and 3, in the simultaneous equations for estimating the b coefficients we have for the 1954-55 season,

$$\begin{aligned} 0{\cdot}0582\, b_1 + 0{\cdot}004\, b_2 - 0{\cdot}015\, b_3 &= \ \ 0{\cdot}138 \\ 0{\cdot}004\, b_1 + 1{\cdot}315\, b_2 - 0{\cdot}077\, b_3 &= \ \ 0{\cdot}515 \\ -0{\cdot}015\, b_1 - 0{\cdot}077\, b_2 + 0{\cdot}040\, b_3 &= -0{\cdot}033 \end{aligned}$$

The solution of these simultaneous equations was obtained by the

TABLE 4

b *coefficients actually used for selection*

Season	Series	Progenies		Coefficients		
		Grown	Selected	b_1	b_2	b_3
1948-49	C(48)	38	12	0·872	1·179	6·958
1949-50	C(49)	44	6	−0·009	0·145	0·232
1950-51	C(50)	30	6	0·170	0·305	1·254
1951-52	C(51)	44	10	0·599	−0·057	2·481
1952-53	C(52)	43	8	−2·475	−1·222	−10·253
1953-54	C(53)	65	9	0·188	0·290	0·444
1954-55	C(54)	58	14	0·236	0·392	0·015

method outlined by Goulden (1939), known as the abbreviated Doolittle method. The coefficients were

$$b_1 = 0{\cdot}236 \qquad b_2 = 0{\cdot}392 \qquad b_3 = 0{\cdot}015$$

which indicates that the selection index should be

$$\mathrm{I} = 0{\cdot}236\, \mathrm{X}_1 + 0{\cdot}392\, \mathrm{X}_2 + 0{\cdot}015\, \mathrm{X}_3$$ where X_1, X_2 and X_3

are separate values for each trait for each progeny and are means of the number of replications employed. Values for the b coefficients for 7 seasons are summarised in table 4.

4. RESULTS

(i) *Basis of expectation*

The expected mean genetic superiority of progenies, selected on the basis of an index in which traits are given optimum weights, is

$$k\sqrt{b_1 g_{1w} + b_2 g_{2w} + b_3 g_{3w}} \cdots \quad . \quad . \quad . \quad (2a)$$

where $k = (\bar{\mathrm{I}}_S - \bar{\mathrm{I}})/\sigma_{\mathrm{I}}$, $\bar{\mathrm{I}}$ is the mean of the selection index of all

progenies, $\bar{I}_S$ is the corresponding mean for selected progenies, and σ_I is the standard deviation of individual values of I. Thus k is the selection differential expressed in terms of standard deviation units.*

Exactly optimum weights would be possible only if *true* values of the g's and t's were known. Since estimates must be employed weights computed are unlikely ever to be optimum though they may be satisfactory approximations. In this case, the expected genetic superiority of selections can be shown to be

$$\frac{k}{\sigma_I}(b_1 g_{1w}+b_2 g_{2w}+b_3 g_{3w})\cdots \quad . \quad . \quad . \quad (2b)$$

As was indicated in the previous section, the computation of the b coefficients in this breeding programme was based on estimates of variances and covariances among progenies within strains. On the other hand selection was actually practised among all progenies without reference to strain. Thus the effective population in which selection occurred was one comprised of progenies belonging to different strains. The effective parameters were those descriptive of the variation of

TABLE 5

Analysis of covariance in bolls per plant (X_1) and yield (X_w) for 1954-55

Variance source	d.f.	Mean product
Replications . . .	3	112·75
Progenies . . .	59	3·07
Plot error . . .	177	2·05
Total . . .	239	...

progenies among, as well as within strains. Consequently, substitution of estimates of $\hat{g}'_{1w}$, $\hat{g}'_{2w}$ and $\hat{g}'_{3w}$ based on the total variation (including that among lines and strains) in equation (2*b*) provides the proper basis for predicting the effect of the selection practised in this programme. The estimates were obtained from analyses of covariance of the form exemplified in table 5 in terms of the data for bolls per plant and yield, from the 1954-55 season. Here the line and strain contributions have not been separated from the total for progenies. The genetic covariance, $\hat{g}'_{1w}$, was estimated as the difference between the mean products for progenies and plot error divided by the number of replications. Thus

$$\hat{g}'_{1w} = (3{\cdot}07-2{\cdot}05)/4 = 0{\cdot}254. \quad \text{Similarly}$$
$$\hat{g}_{2w} = 0{\cdot}350 \text{ and } \hat{g}_{3w} = 0{\cdot}030.$$

* In the case of selection already practised, k can be computed from the data. However, when future selection is being considered, this cannot be done and a probable value is required. The procedure used was to assume normal distribution of the progeny selection index values. With such assumption the expectation of k is given by z/q. Here q is the fraction selected, and z is the height of the ordinate which divides the area of the normal curve into portions q and $1-q$. The required ordinate for a given q can be found from tables of area and ordinates of the normal curve of error such as tables I and II given by Fisher and Yates (1948).

The predicted annual yield increments are given in table 6, together with other data relevant to its estimation.

While the estimates of genetic advance resulting from selection in a single year do not appear impressive, the cumulative estimate shown in the final column is of appreciable magnitude. The results obtained in the progeny bulks and district variety trials provide realistic checks of the actual improvement effected.

(ii) *The behaviour of the modal bulk*

It was thought that bulking of material, " modal " for several traits, would provide a relatively stable check variety which was considered to be essential as a basis for measured advance from selection. The method employed was as follows : after withdrawal

TABLE 6

Expected genetic superiority by seasons. With b *coefficients used and genetic covariances for all progenies*

Season	Series	k	σ_I	$\Sigma b_i g'_{iw}$	Lint/Plant $\bar{X}_w$ gms.	Genetic superiority		
						$\frac{k\Sigma(b_i g'_{iw})}{\sigma_I}$	Per cent.	
							Annual	Cum.
1948-49	C(48)	1·036	2·683	6·543	23·13	2·53	10·9	10·9
1949-50	C(49)	1·429	1·084	−0·005	25·56	0	0	10·9
1950-51	C(50)	1·954	0·719	0·811	17·89	2·20	12·3	23·2
1951-52	C(51)	0·772	2·732	6·651	26·87	1·87	7·0	30·2
1952-53	C(52)	1·352	3·327	−0·246	16·91	0	0	30·2
1953-54	C(53)	1·257	0·477	0·697	22·45	1·84	8·2	38·4
1954-55	C(54)	1·446	0·614	0·197	11·59	0·46	4·0	42·4
					Mean increment			6·0 per annum

of the 23 plants for the 1947-48 progeny rows, the mean and standard deviation of the remaining 397 plants were calculated for the trait lint per seed. All plants deviating from the mean by more than one standard deviation were then eliminated. The mean and standard deviation, for the trait lint length, were then calculated on the plants which remained. Again those deviating by more than one standard deviation were eliminated. There remained 211 plants from which all seed was bulked and this was grown during the 1948-49 season as the first modal bulk (1-MB). Samples of this seed and that from discarded plants were mixed, approximately in the proportion of selected to unselected plants, to provide material which may be designated zero modal bulk (0-MB). This was placed in storage.

Seed for the next modal bulk (2-MB)—and all later modal bulks—was obtained in a slightly different manner. About 500 plants (later

reduced to 300) were selected by field workers. Lint per plant and lint per seed were again recorded for each plant. Plants deviating from the mean by more than one standard deviation were eliminated. Seed weight per seed was then determined on the plants which remained and, after a similar elimination, lint length was estimated on those which remained. This process, always in the same sequence, has been repeated in successive seasons and, contrary to expectation, the mean lint per plant of those which remained after the third process of elimination has, for each season, been higher than the mean of all plants chosen in the field.

The zero modal bulk seed collected in 1947 and placed in storage was grown in the 1953-54 season. The plants were self-fertilised and seed produced was bulked. This fresh seed and a sample of 7-MB were included as extra entries in the replicated progeny row trial of the 1954-55 season. Mean lint yields in grams of lint per plant from

TABLE 7

Comparative yields of selected progenies in bulks trials

Season	Series	Number of replications		Mean lint yield per acre		Increment per cent.
		Locations	Per location	Standard	Selected progenies *	
1949-50	C(48)	1	6	289 (1-MB)	313	8·3
1950-51	C(49)	1	5	233 (2-MB)	245	5·2
1951-52	C(50)	6	3	370 (3-MB)	411	11·1
1952-53	C(51)	4	3	267 (4-MB)	274	2·6
1953-54	C(52)	6	3	244 (5-MB)	267	9·4
1954-55	C(53)	10	3	127 (6-MB)	155	22·0

* All progenies selected during preceding season.

four replications were 9·20 for 0-MB and 12·0 for 7-MB, an advantage of 30·2 per cent. for the latter, or an average of 4·3 per cent. per year.

In 1949-50, both K/51 and 1-MB were included in district variety trials at 13 centres, and in the 1953-54 season, K/51 and 5-MB were entries in progeny bulks trials at six locations. Mean lint yields (in lb. per acre) were as follows :—

Year	K/51	1-MB	5-MB
1949-50	294	289	...
1953-54	335	...	368

Accepting K/51 as a standard of comparison, an increase of 10 per cent. over four generations of the modal bulk is indicated. This amounts to 2·5 per cent. per year as compared to the 4·3 per cent. estimate derived from comparison of 0-MB and 7-MB. These results suggest an effective mechanism of positive selection for yield in the modal bulk population.

(iii) *Yield advance of the progeny row material*

Progeny bulks trials were conducted in six seasons beginning with 1949-50. Numbers of locations in which trials were grown, of replications at each location and mean yields over all locations, are presented in table 7.

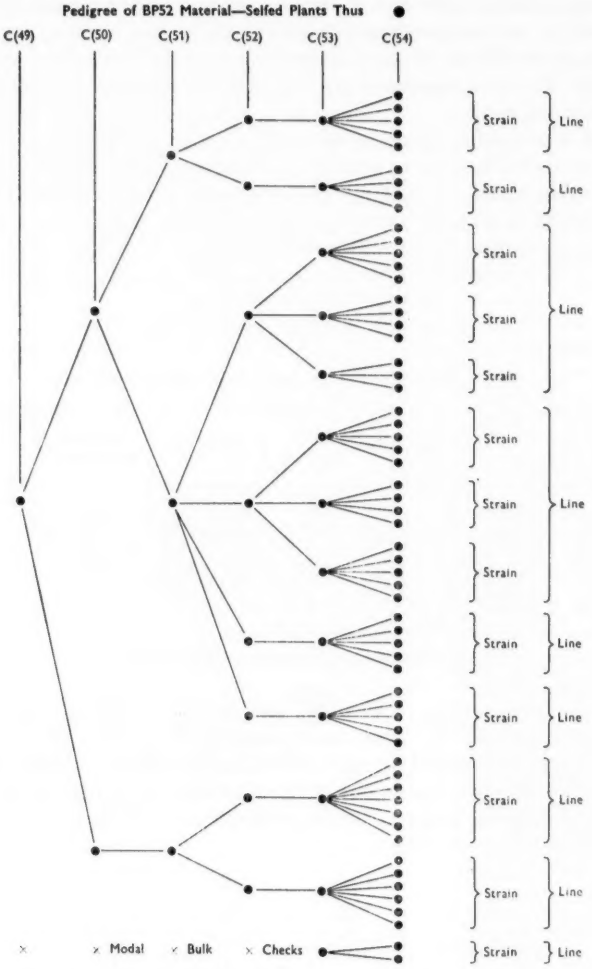

FIG. 1.

Data on mean yields are summarised graphically in fig. 2.

In the expectation that they would remain genetically stable, the successive modal bulks were used as standards of comparison. Since in fact a yield advance has been demonstrated in the modal bulk

series, it is necessary to make an estimate of the annual change in yield of the control series. From the above data it would appear that 3·4 per cent. would be a reasonable figure. The line representing mean yield of the modal bulk standards in fig. 2 is based on this assumption. The points representing yearly means of selected progenies were placed relative to the modal bulk, since the *total* yield advance must be the sum of the improvement in the standard and the amount by which the progenies excelled the standard. For example, in 1954-55 the indicated advance for selected progenies was 22·0 per cent. (the advantage over the 6-MB standard) plus 20·4 (the assumed improvement in the standard itself). Six separate

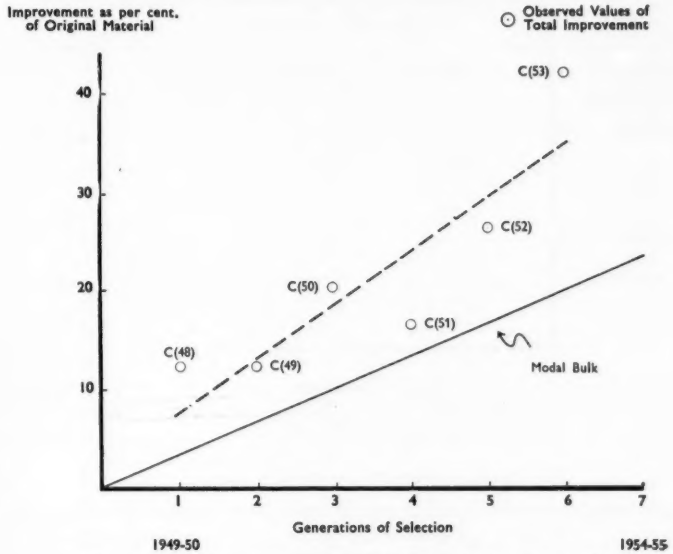

FIG. 2.—Yield improvement of modal bulks and selected progenies over six generations of selection

points are shown and the dotted line is a linear least squares fit to these points. The annual increment, based on the expectation, after six generations of selection, amounts to nearly 6 per cent. This may be compared with the value of 6·3 per cent. per annum derived from the sixth generation in table 6.

District variety trials were conducted in four seasons beginning with 1950-51. A selection to be tested in any series of these trials was grown from second or third generation open-pollinated bulked seed from a progeny selected two or three years previously. Thus the one included in the first series, C(48)12, represents only one generation of progeny row selection. It was grown again in the 1951-52 series. The progeny C(50)20 which reflected three generations

of selection was also included in successive trials. The check variety in these comparisons was K/51. Yield data from these trials are summarised in fig. 3.

The line in this figure represents the linear regression on time of per cent. increment of selected progenies over K/51 and gives an improvement of nearly 20 per cent. after only four generations of selection. This annual increment of nearly 5 per cent. does not differ materially from the 6 per cent. per annum of both the theoretical genetic superiority and that actually achieved from the progeny bulks material.

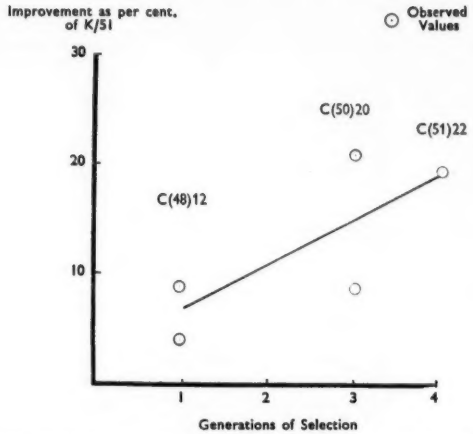

FIG. 3.—Yield improvement of best selected progeny bulk over K/51 Standard in district variety trials

(iv) *Genetic variability in inbred material*

Since the progenies of a single strain trace to a common grandparent and the strains of any single line trace to a common great-grandparent, the variation of both progenies within strains and strains within lines provide evidence on the heterozygosity remaining in such ancestral plants after successive generations of inbreeding. Analyses of variance on the data for the successive seasons provided estimates of the components of genetic variance due to progenies in strains and strains in lines. These estimates are presented in table 8.

With a random choice of parents in each generation, the expected number of heterozygous loci is halved by each generation of self-fertilisation. Hence, assuming absence of interaction between non-allelic genes, (*a*) the expected ratio of progeny to strain genetic variance is one-half, and (*b*) the expected ratio of progeny or strain variance in any year to that in any earlier year is $(\frac{1}{2})^n$ where n is the number of intervening years.

The estimates shown in table 8 do not form a consistent pattern. Averages over the six years in which estimates of both variances were

available, indicate that the ratio of progeny to strain variance was roughly in accord with expectation. However, the trend in magnitude of the estimates is quite evidently not in accord with expectation. Most striking are the results for lint per seed, the trait for which the estimates were actually most reliable. For example, the average estimate of progeny variance in the last two years was about one-third that for the first two years whereas the expected ratio is $(\frac{1}{2})^5$ or 1/32. It should be noted in addition that the progeny variance for this trait was statistically significant in each of the seven seasons.

TABLE 8

Estimates of genetic variance for strains and progenies in strains for traits and yield

Season	Group comparison	Bolls/Plant	Seeds/Bolls	Lint/Seed	Lint/Plant
1948-49	Progenies . .	4·619*	0·082	0·028*	10·604*
1949-50	Strains† . .	2·605*	1·818*	0·094	1·777
	Progenies . .	0	0·436	0·056*	0·871
1950-51	Strains . .	0	5·218*	0	1·227
	Progenies . .	0·273	1·542*	0·055*	0·613
1951-52	Strains . .	8·055*	0·106	0·118*	9·443*
	Progenies . .	3·606*	0·452*	0·105*	3·671*
1952-53	Strains . .	1·832*	2·779*	0·190*	3·947*
	Progenies . .	0	0·142	0·024*	0
1953-54	Strains . .	1·196*	0·768*	0·065*	1·053*
	Progenies . .	0·218	0·536*	0·012*	0·397
1954-55	Strains . .	0	0	0·011	0·186
	Progenies . .	0·118	0·338	0·014†	0·505*
Averages 1949-50 to 1954-55	Strains . .	2·281	1·781	0·080	2·939
	Progenies . .	0·703	0·575	0·045	1·009

* Indicates statistical significance at the 5 per cent. level.

† In all but the first season a strain consisted of all progenies tracing to a common grand-parental plant.

5. DISCUSSION

(i) *Primary selections*

Mason (1938) drew attention to the fact that many of the largest and best established improvements in varietal behaviour in cotton were the result of what he called " primary selections ". Such selections were made by eye judgment in variable field crops, and gave yield increases perhaps of the order of 25 per cent. He compared with these successes the small improvements, laboriously gained and often difficult to demonstrate decisively, from " secondary selection " carried on by orthodox breeding methods on experiment stations.

It is in the nature of variability that it should be possible to pick out from an unselected, highly variable crop, components differing widely from the mean. Often, and especially when a crop is comparatively new in an area, or is exposed to such new circumstances as attacks from pests or diseases not previously encountered, selections may be made that will be vastly superior to the unselected material. What is not so generally realised is that the slow, patient, progressive improvement of an already well adapted stock may over the years yield a greater return than a single spectacular stride, and, moreover, that what is known of evolutionary change suggests that this kind of improvement is more comparable with natural evolutionary progress than is the quick response of a successful primary selection.

One of the essential objects of the breeding programme under review was to assess the practicability of breeding for a rather small, but progressive, improvement over a period of years in a stock in which all the major possibilities of advance had already been exploited. Since all the BP52 material traced to a single plant, there was, in 1945, reason to question whether it contained enough genetic variation to provide the basis for significant yield improvement. In fact, however, data from large scale field and district trials indicate a measured yield improvement at the rate of from 5 to 6 per cent. per annum, resulting in a total improvement of some 30 per cent. after six generations of selection.

(ii) *Selection index*

The predicted improvement (table 6) from six generations of selection was 38 per cent. as compared with the estimate of 35 per cent. provided by the linear regression in fig. 2. This was based on comparisons with the modal bulk checks. District variety trials, conducted over a much wider range of environmental conditions, indicate an annual improvement, relative to K/51, of approximately 5 per cent. (see fig. 3). In view of the seasonal variation in climatic conditions that occurred, the close approach of the measured to predicted improvement is very impressive. The evidence from the district variety trials is especially encouraging since there is always reason to question whether material, selected at a specific location, will perform at the same high level over an entire area and some variation in management practice.

There is some question regarding the optimum use of data available at any given time. The index was calculated each season entirely on the data pertaining to progenies raised in that season. Alternatively the index might have been adjusted each year in terms of all the accumulated data. The argument for using data from the particular season is that the most effective weighting of several traits is a function of the exact environmental conditions in which the crop was raised. It is well known that genetic variation in a specific trait may be exhibited more clearly in some years than others. This point may be

demonstrated in another way by considering the expectation of an estimate of genetic covariance in detail. A single plot value may be represented as

$$P_{jkm} = \mu + g_j + f_k + (gf)_{jk} + e_{jkm}$$

where μ is the population mean over all years,
g_j is the average genotypic effect for the j-th progeny,
f_k is the effect of the environment of the k-th season,
$(gf)_{jk}$ is the effect of interaction between the j-th progeny and the environment of the k-th season, and
e_{jkm} is a random effect (experimental error) associated with the plot on which the j-th progeny is grown in the m-th replication.

Working from this model, the expectation of the estimate of the genetic covariance between the i-th trait and lint yield is found to be

$$\sigma g_i g_w + \sigma(gf)_i g_w + \sigma g_i (gf)_w + \sigma(gf)_i (gf)_w \quad . \quad (3)$$

The optimum b coefficients would be obtained by using

$$g_{iw} = \sigma g_i g_w + \sigma(gf)_i g_w.$$

To estimate $\sigma g_i g_w + \sigma(gf)_i g_w$ separately from $\sigma g_i (gf)_w$ and $\sigma(gf)_i (gf)_w$ requires data that are beyond the scope of a practical breeding programme. The procedure actually followed estimates the quantity given in equation (3) above. On the other hand, pooling the accumulated data would have provided estimates of the quantity $\sigma g_i g_w$ plus the mean of the remaining three elements of equation (3) for the seasons for which the data were pooled. This mean would approach zero as the number of seasons was increased. Neither procedures provide exactly what is desired nor is it possible to state with certainty which is better. However, the pooling approach abandons the hope of capitalising on the specificity of the particular season.

An issue of practical importance is the cost in time and effort required for scores or measurements on which the selection index is based. In particular, boll counts are very time consuming and are difficult to obtain with precision. Counts made at a particular time, that must precede the first reaping, will not necessarily include all bolls that may develop soon enough to contribute to the final reaping. The effort involved in repeated counts would be prohibitive. On the other hand, seeds per boll and lint per seed are evaluated at considerably less expense and, in addition, measurement errors are more likely to be detected by routine checks. Fairfield Smith (1936) pointed out that when yield is completely determined by two or more component traits, all available information can be incorporated in the index in terms of either the full set of component traits or by substitution of yield for any one of them. In view of the manner in which measurements were made in this work, yield was not completely

specified by the data for the three fundamental yield components and, therefore, a slightly more effective index might have resulted from inclusion of lint yield itself. However, the point made by Fairfield Smith (1936) strongly suggests that use of lint yield and any two of the three component traits would supply most of the information available from the three traits and lint yield in this study. Because of the high cost of satisfactory boll counts, it is proposed to use an index based on seeds per boll, lint per seed and lint yield in future selection indices. An illustration of how such an index may compare with that actually used in the past, is provided by computation of the expected genetic superiority associated with these two indices in the case of the 1954-55 data. For the index involving bolls per plant, the expected genetic superiority was computed as 4·0 per cent. (table 6) and for the index in which lint yield is substituted for boll number, the comparable figure was 5·4 per cent. In other cases the advantage might not be as great but there is no reason to doubt that lint yield would be at least as effective as boll number.

In the genetic improvement of any crop plant the breeder is always faced with the problem of weighting the wide variety of traits which, in his opinion, have a bearing on the utility of the plant. Almost universally this weighting has been subjective with a great deal of reliance on intuition. The selection index approach provides an objective basis for determining the optimum relative weights to be accorded any combination of characters. It requires only that a quantitative measure is provided for each character. The *b* coefficients indicate the proper attention to be given each and if it approximates to zero, this is not to infer that the character itself is of no physiological significance whatsoever. What it does mean is that the appropriate attention to the trait is provided indirectly through the emphases accorded to the related traits. The approach outlined here appears apposite as a basis for incorporating resistance to bacterial blight into the Uganda selection programme.

While evidence has been presented for persistence of genetic variability within continuously self-fertilised lines of cotton, it is now unlikely that the magnitude of such variation would justify a continued major programme of selection within inbred lines. On the other hand this persisting genetic variation provides the opportunity for effecting adjustments of minor weaknesses in otherwise superior strains. Moreover, a panmictic population, derived from inter-crosses of selection lines, should exhibit nearly the same order of genetic variability that was demonstrated by BP52 itself. Such a population should reflect the effect of selection practised thus far and therefore provide a higher base to build through future selection.

(iii) *Persistence of genetic variability*

Continuous self-fertilisation is widely used with diploid organisms for developing lines within which there is a minimum of genetic

variation. New World cottons, though amphidiploid in origin, are usually considered to function as diploids. Thus a question arises concerning the utility of selection at all within lines that have resulted from more than a few generations of inbreeding. The present data are not the first suggestion that appreciable genetic variation may persist in the face of continuous self-fertilisation of cotton. In this connection Hutchinson (1940) commented on the failure to achieve "pure lines" in Sea Island cotton. Harland (1934) reported that after 13 generations of self-fertilisation, selection for lint index (lint per seed) in the Montserrat Sea Island variety of cotton still led to yield improvement. Similar results were obtained by the writer in 1944 using material of a hybrid between the Montserrat and St Vincent Sea Island varieties. After five generations of inbreeding, during which time selection intensity was low, a new programme was designed to direct intense selection in three directions. In one population, selection pressure was exerted in favour of boll number, ignoring other traits and, in another, similar pressure was exerted in favour of the trait lint per seed. In a third, selection was based on yield only. This procedure was followed for the next six seasons always using self-fertilised material for selection. At the end of this period estimates of genetic variances for progenies in strains were as follows :

Objective in selection	Genetic variance for traits	
	Bolls per plant	Lint per seed (CGM)
High boll number . .	15·597*	0·0223†
High lint per seed . .	0·339	0·1076*
Yield only . . .	5·912	0·0052

* $p = 0{\cdot}01$ † $p = 0{\cdot}05$.

As in the case of the comparable Uganda data, the observed variances are considerably in excess of the residual variances which might be expected in a functional diploid after the same indicated number of generations of inbreeding. It is of interest to note that the largest estimates of genetic variance in individual traits were obtained in populations in which selection pressure was confined to the trait in question. Such extremes were not observed in the St Vincent population in which selection was for yield (a composite of several traits), or in the Uganda material where the selection criterion gave some weight to each of three characters.

There are a number of possible explanations for persistence of variability in inbred cotton, one of which is undoubtedly that with the statistical techniques that were used, it was possible to detect those strains in which there was sufficient genetic variation to provide

scope for further improvement. The problem is further discussed elsewhere (Manning, 1955). It is sufficient here to emphasise that genetic variation of a magnitude to lead to practical yield improvement has been demonstrated to exist after seven generations of inbreeding, and there is some evidence for the view that a breeding programme can be so designed as to conserve the variability necessary for further improvement.

(iv) *Selection in the modal bulk*

The genetic improvement demonstrated for the modal bulk population indicates that, contrary to the original intent, some form of selection pressure for yield must have been operative. It is of interest to speculate on the ways in which this may have occurred. At least three possibilities can be envisaged.

Season	Selection differential (per cent.)
1947-48	7·8
1948-49	3·9
1949-50	3·1
1950-51	0·1
1951-52	1·2
1952-53	6·2
1953-54	6·3
Average	4·0

It will be recalled that there were actually two stages of selection. The first consisted of taking a sample of 300 to 500 plants from a field of some 6000 plants. Secondly, the modal selection practised in the sample of plants actually taken from the field itself, resulted in a positive selection differential for yield. The differences in the mean lint yield of the plants from which seed was actually used, and of the entire sample from which they were taken, are listed above in per cent. of the mean for the entire sample.

The mechanism by which these differences arose is uncertain but either non-linear relations between yield and the traits on which the modal selection was based, or asymmetrical distribution of one or more of the latter, are indicated. Unless the heritability of yield in single plants was very considerably more than it is reasonable to suppose, a selection differential of no more than 4 per cent. could have contributed only a small part of the annual genetic improvement previously indicated.

Finally, the effects of automatic selection incidental to the close relationship between seed number and yield would be a contributing factor of some importance.* The effective selection differential above

* This explanation was put forward by Dr R. E. Comstock of the Institute of Statistics, North Carolina State College.

$\bar{Y}$, the unweighted mean for plants from which seeds were actually bulked would be computed as $\Sigma XY/\Sigma X - \bar{Y}$ where X is number of seeds per plant, Y is yield per plant, and summation is over all plants. It may be estimated on the basis of the following argument. Assuming linear regression of Y and X, which was clearly demonstrated in the data,

$$Y = \bar{Y} + \beta_{YX}(X - \bar{X}) + \text{a random deviation from regression.}$$

Then integrating $XYf(X)$ and $Xf(X)$ over the range of the distribution

$$\bar{Y}\bar{X} + \beta_{XY}\sigma_X^2 \quad \text{and} \quad \bar{X}$$

are obtained. Dividing the former by the latter

$$\bar{Y} + \frac{\beta\sigma_X^2}{\bar{X}}$$

is obtained as the expectation of $\Sigma XY/\Sigma X$. Substituting estimates of the several parameters, the selection differential in per cent. of the mean is approximated as 12 or 13 per cent. Values of the selection differential as outlined were compared with actual values of

$$(\Sigma XY/\Sigma X) - \bar{Y}$$

computed from the single plant data for two years. The agreement was satisfactory. Since the automatic selection differential was in addition to that resulting from the modal selection, estimates of the two may appropriately be summed to obtain a total estimate of 16 or 17 per cent. Assuming a heritability (on the single plant basis) of from 10 to 15 per cent., which is not incompatible with the estimates of genetic variance in the inbred material and the phenotypic variance of single plants, this would allow for an annual improvement of from 1·7 to 2·55 per cent. This compares with the 3·4 per cent. indicated by yield trials, a portion of which may have resulted from the field selection of the sample in which modal selection was practised.

6. SUMMARY

1. A Selection Index technique was employed to bring about yield improvement in self-fertilised material of the BP52 Upland variety of cotton grown extensively in Uganda.

2. Genetic advance of the order of 35 per cent. was recorded after six generations of selection.

3. It was inferred that considerable genetic variability must have been present in the original material. Moreover, its persistence in the face of self-fertilisation provides a justification for continued selection within inbred material.

4. While only the primary components of yield, namely, bolls per plant, seeds per boll and lint per seed were actually used as the basis for the selection index in this work, attention is drawn to the fact that additional factors may be included for any traits which appear truly different to the eye.

5. The improvement in the modal bulk check standard suggests that natural selection favours the breeder whenever number of seeds produced is intimately related to yield.

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THE GENETICAL CONSEQUENCES OF ASSORTATIVE MATING

E. LESLIE BREESE

Agricultural Research Council Unit of Biometrical Genetics, Department of Genetics, University of Birmingham

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1. INTRODUCTION

THE disposition of the heritable portion of continuous variation under different systems of mating is of considerable interest both to the geneticist and to the plant or animal breeder. For many of the more usual experimental mating systems the behaviour of such variability can be predicted with some precision (*e.g.* selfing, random and sib-mating). Assortative (*i.e.* like to like) mating has, however, in the past received less attention. Wright (1921) has pointed out that, provided the number of genes involved is large, such a system is unlikely to achieve a greater *genetic correlation* (*i.e.* correlation between allelic genes) than is obtained under random mating. Hence, initially little increase in homozygosity would be achieved compared with a system of random mating. But at the same time we should expect an increased association between parents for genes having the same overall potential, an increase in what may be termed the *genotypic correlation*, and it is in the consequences of such a correlation that our present interests lie.

Experiments were devised therefore to investigate within a common parental population changes in heritable variation effected by assortative mating (*A*) as compared with selfing (*F*) and random mating (*R*) systems. Mather (1949*a* and *b*) has described how the heritable portion of continuous variation may be partitioned into various components and the way in which these may be apportioned to fit certain experimental populations. These methods have been used to describe and assess changes in heritable variation during the course of inbreeding experiments (Bateman and Mather, 1951 ; Mather and Vines, 1952 ; Hayman, 1953), and they can be extended to deal with assortative mating and so serve as a basis for comparing the genetical consequences of the three mating systems under consideration.

2. DISTRIBUTION OF VARIABILITY

In a randomly breeding population the variation exhibited by any continuously varying character may be partitioned into three components, provided that genic interaction be neglected. These are D, measuring the fixable component, H, depending on the departure of the heterozygote from the mean of the two corresponding homozygotes, and E, measuring non-heritable variation. Where each gene is represented by two allelomorphs having equal frequencies, the

variance of the individuals within such a population may then be expressed as $\frac{1}{2}D+\frac{1}{4}H+E$ (see Mather, 1949*a* and *b*).

The three different types of progeny which can be obtained from a randomly breeding population by imposing the mating systems with which we are concerned, each yield three second degree statistics. These are (1) the variance of family means (1st rank variances), (2) the mean variance within families (2nd rank variances), and (3) the covariance of family means and parental mean values. Following the biometrical method described by Mather, the composition of the various statistics may be derived in terms of D, H and E. In the case of *F* and *R* generations the expectations have already been formulated and are reproduced for purposes of comparison in table 1. The derivation of corresponding expectations for the *A* generation, however, involves further basic premises to which we must now turn.

Consider two genes *A*-*a* and *B*-*b* where the capital letter denotes the allele giving greater expression of the character in question. Assuming the absence of dominance and interaction, and that genes are equal in effect (i.e. $da = db$; $ha = hb = 0$), the nine possible genotypes in a randomly breeding population can be assorted phenotypically into five intramating groups provided that environmental fluctuations are negligible. These are (1) *AABB*, (2) *AABb* ; *AaBB*, (3) *AAbb* ; *AaBb* ; *aaBB*, (4) *aaBb* ; *Aabb*, and (5) *aabb*, and will occur in the ratio 1 : 4 : 6 : 4 : 1 respectively. Letting d_a and d_b measure the departure of *AA* and *BB* ; and $-d_a$ and $-d_b$ the departure of *aa* and *bb* from the mid-parent, the variance of means of families derived from these mating groups will be

$$\tfrac{1}{4}(d_a{}^2+d_b{}^2)+\tfrac{1}{8}(d_a+d_b)^2.$$

When *n* genes of equal effect are considered, this term may be expressed as

$$\tfrac{1}{4}\Sigma d_a{}^2+\frac{1}{4n}(\Sigma d_a)^2 = \tfrac{1}{2}G,$$

where G denotes the prospective genetic variation in the inbred (homozygous) population assuming equal frequencies and independent assortment of the genes.

By comparison, the variance of *R* family means is $\frac{1}{4}\Sigma d_a{}^2(=\frac{1}{4}G)$, so that assortatively mating the parents increases the genetic variation between families by $\frac{1}{4n}(\Sigma d_a)^2$, provided that genotypic correlation is complete. Expansion of this term indicates that only $\frac{1}{4n}$ of this increased variance is contributed by the independent deviations of homozygotes from the operative mean (expressed as $\Sigma d_a{}^2$ and corresponding to Mather's D) the remaining $\frac{n-1}{4n}$ being dependent upon product terms $d_a d_b$, etc. deriving from an excess of reinforcing homozygotic/homozygotic gene combinations (*AABB* and *aabb*, where two

The distribution of heritable variation following a single generation of selfing (**F**) *assortative mating* (**A**) *or random mating* (**R**), *assuming random mating and equal gene frequencies in the parental population*

Stat.	Mating system	No. of genes	No dominance $d_a = d_b$; $h_a = h_b = 0$			Reinforcing dominance $d_a = d_b = h_a = h_b$							Opposing dominance $d_a = d_b = h_a = -h_b$						
			D	D′	G	D	H	D′	H′	B	B′	G	D	H	D′	H′	B	B′	G
1st rank variance	*F*	n	$\frac{1}{2}$	...	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{16}$	...	...	...	...	$\frac{9}{16}$	$\frac{1}{2}$	$\frac{1}{16}$	...	...	...	...	$\frac{9}{16}$
	A	1	$\frac{1}{2}$	...	$\frac{1}{2}$	$\frac{5}{12}$	$\frac{1}{18}$	...	...	$\frac{1}{6}$	...	$\frac{23}{36}$	...	...	...	...	...	...	$\frac{23}{36}$
		2	$\frac{3}{8}$	$\frac{1}{8}$	$\frac{1}{2}$	$\frac{1}{3}$	$\frac{19}{288}$	$\frac{1}{12}$	$\frac{1}{48}$	$\frac{1}{12}$	...	$\frac{169}{288}$	$\frac{11}{30}$	$\frac{101}{480}$	$\frac{1}{10}$	$-\frac{3}{10}$	$\frac{7}{60}$	$\frac{1}{15}$	$\frac{317}{480}$
		n	$\frac{n+1}{4n}$	$\frac{n-1}{4n}$	$\frac{1}{2}$	...	...	...	...	...	...	$\frac{69+31/n}{144}$	...	...	...	...	...	...	...
	R	n	$\frac{1}{4}$	...	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{16}$	...	...	...	...	$\frac{5}{16}$	$\frac{1}{4}$	$\frac{1}{16}$	...	...	...	...	$\frac{5}{16}$
2nd rank variance	*F*	n	$\frac{1}{4}$	...	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{8}$	...	...	...	...	$\frac{3}{8}$	$\frac{1}{4}$	$\frac{1}{8}$	...	...	...	...	$\frac{3}{8}$
	A	1	$\frac{1}{4}$	...	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{6}$	...	...	$-\frac{1}{6}$	...	$\frac{1}{4}$	...	...	...	...	...	...	$\frac{1}{4}$
		2	$\frac{1}{4}$	...	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{17}{96}$	...	...	$\frac{1}{12}$	...	$\frac{49}{96}$	$\frac{1}{4}$	$\frac{83}{480}$	...	...	$-\frac{7}{60}$	...	$\frac{147}{480}$
		n	$\frac{1}{4}$	...	$\frac{1}{4}$	...	...	...	...	...	...	$\frac{7-3/n}{16}$							
	R	n	$\frac{1}{4}$	...	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{3}{16}$	...	...	...	...	$\frac{7}{16}$	$\frac{1}{4}$	$\frac{3}{16}$	...	...	...	...	$\frac{7}{16}$
Covariance	*F*	n	$\frac{1}{2}$	...	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{8}$	...	...	...	...	$\frac{5}{8}$	$\frac{1}{2}$	$\frac{1}{8}$	...	...	...	...	$\frac{5}{8}$
	A	1	$\frac{1}{2}$	...	$\frac{1}{2}$	$\frac{5}{12}$	$\frac{1}{12}$	...	...	$\frac{1}{6}$	...	$\frac{2}{3}$	...	...	...	...	...	...	$\frac{2}{3}$
		2	$\frac{3}{8}$	$\frac{1}{8}$	$\frac{1}{2}$	$\frac{1}{3}$	$\frac{1}{24}$	$\frac{1}{12}$	...	$\frac{1}{12}$	$\frac{1}{24}$	$\frac{7}{12}$	$\frac{11}{30}$	$\frac{37}{240}$	$\frac{1}{20}$	$-\frac{11}{120}$	$\frac{7}{60}$	$\frac{7}{120}$	$\frac{157}{240}$
		n	$\frac{n+1}{4n}$	$\frac{n-1}{4n}$	$\frac{1}{2}$	...	...	...	...	...	...	$\frac{3+1/n}{6}$	...	...	...	...	...	...	...
	R	n	$\frac{1}{4}$	...	$\frac{1}{4}$	$\frac{1}{4}$	...	...	...	...	...	$\frac{1}{4}$	$\frac{1}{4}$	...	...	...	...	...	$\frac{1}{4}$

genes only are considered). This part of the variation we can denote by D′.

The variance of *A* family means can now be re-expressed as

$$\frac{n+1}{4n}\,\mathrm{D}+\frac{n-1}{4n}\,\mathrm{D}'=\tfrac{1}{2}\mathrm{G}.$$

In the same way terms may be obtained for the 2nd rank variance and the covariance.

The constitution in terms of D and D′ of the three statistics for all three mating systems are given in table 1 for 1, 2 and *n* genes assuming no dominance. No D′ items occur in the *F* and *R* statistics, of course, since the product terms contributed by reinforcing hom/hom combinations cancel out against corresponding negative terms derived from equal proportions of opposing hom/hom combinations (i.e. *AAbb*, *aaBB*, etc.). It will be noted that although the proportion of the genetic variability exhibited by *A* family means remains for any number of genes statistically equivalent to that obtained under selfing, its constitution in terms of D and D′ depends on the number of genes (*n*) involved. Where *n* is large the proportion of G exhibited as D is not appreciably greater for assortative than for random mating; the increased genetic variation therefore depending not on increased homozygosity but on an excess of reinforcing, and a corresponding deficiency of opposing, hom/hom combinations. Continued perfect assortative mating should thus result in an accumulation of reinforcing hom/hom combinations and finally achieve fixation of genetic variability in the free state (see also Wright, 1921).

For all mating systems and for any number of genes the within family variances remain constant as $\frac{1}{4}$D. The covariance formulæ are, however, equivalent to those of their corresponding 1st rank variances and as such are valuable additional statistics for the estimation of variability components.

Since the effects of assortative mating ultimately depend on the precision whereby potential parents may be assigned to particular genotypic intramating groups, any factor influencing the expression of the character, genetic or environmental, must affect the consequences of the system. Thus, for instance, full dominance of the genes concerned, by preventing the separate classification of the heterozygote, alters the composition of intramating groups. Allowing explicitly for these effects, however, involves further assumptions regarding the properties of individual gene pairs. Expectations so derived, therefore, are likely to have but limited use in general analysis since each formulation must be applicable only to the situation as defined. Nevertheless the general implications may be discovered by taking two simple examples.

First we can arbitrarily assume that dominance is complete for all genes and that the direction is the same in each case (i.e. $d_a = d_b = h_a = h_b$, etc.). Then if two genes are considered the nine possible

genotypes of a randomly breeding population form three intramating groups when genotypic classification is complete. The expectations for all three statistics may be derived as before and are given together with the comparable F and R statistics in table 1. The proportion of G, given these conditions, has been calculated for n genes (where $n > 1$) by Dr Hayman of this Unit, and is also given in table 1. Consideration of the two gene situation indicates that the increase in genetic variation of family means compared with that obtained under random mating now depends not only on D′ but also on H′ ($h_a h_b$, etc.) and B ($d_a h_a$; $d_b h_b$, etc.) items. These latter items measure the average contribution of individual genes to that proportion of the variability deriving from an excess of reinforcing heterozygotic/heterozygotic and homozygotic/heterozygotic combinations. In addition, where the number of genes involved is large, the total genetic variation is now lower than that expected under selfing. Even so, the frequencies of genic combinations giving extreme effects are being increased at the expense of the more balanced combinations, and continued perfect assortative mating must again ultimately result in the fixation of extreme expressions of the character in question. However, compared with the situation involving no dominance, the process must be considerably retarded, at least at that end of the distribution towards which dominance is directed.

The covariance statistic, although including B′ ($d_b h_a$, $d_a h_b$, etc.) in place of an H′ item, still depends for its increased value relative to the R generation on items arising from reinforcing genic combinations and as such is again a useful additional statistic in estimation. The 2nd rank variance is now no longer equivalent in its constitution to that of the R generation : but where n is large the two statistics should not differ in value and so this statistic can play little part in comparisons between mating systems.

Finally, in table 1, equations for the statistics have been derived for two genes where dominance is complete but opposing. The presence of positive D′, B and B′ and negative H′ items in the 1st rank statistics again indicates an excess of reinforcing genic combinations and hence essentially a situation similar to that observed for reinforcing dominance. By contrast, however, the 2nd rank variance expectation, where $n = 2$, is lower than is obtained under random mating ; but although expectations involving larger numbers of genes have not been derived, it must be expected that as n increases the nearer will the proportion of genetic variability displayed by this statistic approach that under random mating.

More complex genetical situations involving genic interaction and linkage have not been considered. But whatever the genetical control of the character, it cannot but be inferred that the continued reassociation of genes into combinations having extreme effects at the expense of more balanced combinations will remain the most important attribute of assortative mating ; and while dominance, genic inter-

action or linkage in opposition can retard the process, continued perfect assortative mating must eventually achieve fixation of the extreme expressions of the character involved unless true overdominance exists.

Although in theory the generation to generation increase in variance of *A* families relative to *R* families can be related to different additional components of variation, depending on the genetic control of the character, the recognition of these components separately is likely to prove difficult. With data from but a single generation available, the overall effects of assortative mating can best be measured by estimating any increase in genetic variation of *A* families relative to that expected under random mating, using both *F* and *R* generations as baselines for assessment. *F* and *R* generations can also be employed in determining the degree of dominance and other genetic effects in the data, and any detected increase in genetic variation in the *A* generation may then be interpreted accordingly. Doubtless some direct separation of the components entering into the increased variance could be effected by suitable experiments but so far this has not been attempted.

3. THE EXPERIMENTS

(i) *Structure*

The investigations involved the setting up of two experiments, basically of the same design but differing in the numbers of parents involved and the material used. In both instances plants used in the formation of a parental pool were individuals of F_2 populations derived from crosses between inbred lines of *Nicotiana rustica*. It may safely be assumed therefore that the basic individuals were fairly representative in terms of average heterozygosity of a randomly breeding population : a population, moreover, wherein each gene was represented by two allelomorphs only, each having a frequency of 0·5.

The choice of a quantitative character, upon which the effects of assortative mating could be studied, presented little difficulty. Flowering time was the most obvious in that data needed for the construction of the mating programme could be collected early enough in the season to ensure that all crosses could be successfully completed during the same season.

In 1951, F_2 plants derived from a cross between our varieties 1 and 5 of *Nicotiana rustica* (see Mather and Vines, 1953) were grown in two blocks each consisting of 50 individuals. Plants were then assortatively and randomly mated on a between block basis in the following manner. Firstly the earliest flowering plant in Block I was mated to the earliest flowerer in Block II, the second earliest in Block I to the second earliest in Block II, and so on, giving a total of 50 independent assortative pair matings and yielding a possible 50 families of the third generation (A_3). Secondly, plants were mated

according to their field position, the first plant in Block I to the first in Block II, the second to the second, etc. Since individuals within the blocks were planted out at random (*i.e.* taken as they came from seed pans and seed boxes) field position should have been independent of genotype, and this therefore yielded 50 families derived by random mating in the third generation (R_3). (In this instance the R_3 generation corresponds to an S_3 generation under the notation of Mather and Vines.) In addition 100 F_3 families were obtained by selfing each individual of the parental pool.

In practice, 48 families of each biparental generation and 99 F_3 families were available for sowing in 1952 and these, with the parents, F_1 and F_2 were grown in a randomised two-block layout of a design similar to that devised by Mather and Vines.

The 1953 experiment was initiated in 1952 using individuals of an F_2 population stemming from a cross between varieties 12 and 2 of *Nicotiana rustica*, but 40 plants only were used in the construction of a parental pool. Twenty A_3, 20 R_3 and 40 F_3 families obtained by the methods described above were available for sowing in 1953 and these were grown in essentially the same experimental layout as that of 1952.

In both cases the plants were scored for flowering time (expressed in days after 20th June) and also for final height (in inches).

(ii) *Correlation between parents for flowering time and height*

An indication of the success of the methods employed in randomising and assorting the parents can be obtained by calculating the correlation coefficient (r) between plants forming the male and female parental series of the A and R systems respectively. The appropriate r values for flowering time are given in the first part of table 2 together with the probability of the departure of these values from zero. For both experiments only the A male and female series show significantly high values, the R values indicating nothing but true random association. In both cases, therefore, the requisite degrees of phenotypic correlation have been achieved, although in the 1952 experiment the degree of correlation ($r = 0{\cdot}86$) was somewhat lower than expected.

Mather and Vines note that flowering time and height appear to be partially correlated in expression in so far as the taller plants tend to be later flowering. The between character r values calculated for 120 F_2 plants associated with the 1952 experiments and 100 F_2 plants associated with the 1953 experiments show such to be indeed the case (see table 2). Accordingly parental correlations in respect of height were found for the different mating systems in both experiments and these are also shown in table 2. It will be observed that in the 1952 experiment assorting on flowering time resulted in a significant correlation between mates with regard to height, although the strength of this correlation is considerably lower than for the primary character itself. Despite a similar correlation between height

and flowering time as in the 1952 experimental material, parental correlation for height was not achieved in the 1953 experiment. The bias extended to height as a partially correlated character by assorting on flowering time depends, of course, in a statistical manner on the strength of correlation between characters and this correlation was sufficient to achieve a significant association of plants in respect of height in but one instance.

Analysis of the height data in addition to the flowering time data should, therefore, provide us not only with information as to the effects of different levels of assortative mating, strong and weak, but also give some indication of the degree and nature of any correlated effect which may be induced in height as a secondary correlated character.

TABLE 2

Correlation between parents for flowering time and height, 1952 and 1953

Parental correlation between	1952			1953		
	Correlation coeff. (r)	N	Dev. from o P	Correlation coeff. (r)	N	Dev. from o P
Flowering time						
A ♂ and ♀ series	0·86	46	<0·001	0·97	18	<0·001
R ♂ and ♀ series	−0·09	46	...	0·29	18	>0·1
Height						
A ♂ and ♀ series	0·38	46	0·01-0·001	0·19	18	>0·1
R ♂ and ♀ series	0·18	46	>0·1	0·00	18	...
Height and flowering time						
Height and flowering time	0·45	118	<0·001	0·47	98	<0·001

4. ANALYSIS OF VARIABILITY

(i) *General*

For each of the two characters and each of the three mating systems, three second degree statistics have been calculated and are given in tables 4 and 5 together with their compositions in terms of G and E (the environmental component), assuming no dominance. The notation devised by Mather and Vines (1953) to denote the different statistics has been extended to cover present requirements and is presented in table 3.

The direct estimates of E_1 and E_2 were obtained by the methods of Mather and Vines except in the case of the E_2s of 1953. In this

year E_2s were obtained from F_2 plot means and not F_1 and parental plot means. It was considered that this would allow not only for

TABLE 3

Second degree statistics calculated for progenies

Notation	
$V1F_2$	Variance of means of families derived by selfing
$V2F_2$	Mean variance of families derived by selfing
$W1F_{22}$	Covariance of family means and parental values
$V1A_2$	Variance of means of assortative biparental progenies
$V2A_2$	Mean variance of assortative biparental progenies
$W1A_{22}$	Covariance of the family means and the means of the parental pair values
$V1R_2$, $V2R_2$, $W1R_{22}$	Analogous to above statistics but for the progenies of random biparental matings

TABLE 4

Results—height and flowering time—1952

Statistic	Composition (H = 0)	Observed			Expected
		I	II	Mean	
Flowering time					
E1	E_1	5·2449	7·3301	6·2875	18·3784±0·8767
E2	E_2	2·1004	4·5154	3·3079	7·6888±0·9516
$V1F_2$	$\frac{1}{2}G+E_2$	34·2642	25·2843	29·7743	26·5941±1·0364
$V2F_2$	$\frac{1}{4}G+E_1$	25·2120	24·0040	24·6080	27·8310±0·7882
$W1F_{22}$	$\frac{1}{2}G$	12·0108	12·5519	12·2814	18·9053±1·0860
$V1A_2$	$\frac{1}{2}G+E_2$	21·7435	23·0494	22·3965	19·7130±1·1967
$V2A_2$	$\frac{1}{4}G+E_1$	42·5375	43·4267	42·9821	27·8310±1·0158
$W1A_{22}$	$\frac{1}{2}G$	7·9965	10·6846	9·3406	12·0242±1·1967
$V1R_2$	$\frac{1}{2}G+E_2$	14·8714	16·4438	15·6576	17·1414±0·8336
$V2R_2$	$\frac{1}{4}G+E_1$	27·9854	28·0000	27·9927	27·8310±0·7882
$W1R_{22}$	$\frac{1}{4}G$	6·3074	5·1554	5·7314	9·4526±0·5430
Height					
E1		4·8937	5·7968	5·3453	10·5030±1·0045
E2		3·8950	4·6749	4·2850	6·0749±1·1337
$V1F_2$		23·3099	22·3106	22·8103	21·6200±1·2347
$V2F_2$		20·4859	17·6428	19·0644	18·2755±0·9390
$W1F_{22}$		12·3073	12·6316	12·4695	15·5451±1·2938
$V1A_2$		16·9347	18·4715	17·7031	15·9594±1·4257
$V2A_2$		16·5208	26·3611	21·4410	18·2755±1·2102
$W1A_{22}$		8·4733	7·8085	8·1409	9·8845±1·4257
$V1R_2$		10·5538	14·8513	12·7026	13·8474±0·9932
$V2R_2$		17·1417	21·8146	19·4782	18·2755±0·9390
$W1R_{22}$		7·4326	7·6251	7·5289	7·7726±0·6469

field variation but also to some extent for sampling variation entailed in the use of segregating families. Doubtless a more satisfactory

method would have been to have used E_2s estimated in the normal manner and to have introduced a separate term for sampling variation based on the numbers of plants per plot and the within family variance: but at the time it was not considered that the extra computation involved would have been justified.

Information as to the change in heritable variation associated with different mating systems could be obtained by a modified form of offspring/parent regression analysis. But more useful and more

TABLE 5

Results—height and flowering time—1953

Statistic	Observed			Expected
	I	II	Mean	
Flowering time				
E1	24·9895	36·8400	30·9148	34·6627±4·8571
E2	25·5500	45·1980	35·3740	34·1481±5·2718
$V1F_2$	127·0143	136·9804	131·9974	126·5178±9·7473
$V2F_2$	74·6950	65·9225	70·3088	80·8475±6·7980
$W1F_{22}$	80·8909	78·9900	79·9405	92·3697±6·0162
$V1A_2$	85·6994	76·3506	81·0250	90·6124±9·9767
$V2A_2$	68·7700	94·4100	81·5900	80·8475±6·7980
$W1A_{22}$	63·7726	68·3308	66·0517	56·4643±6·6295
$V1R_2$	94·8475	71·5815	83·2145	80·3329±7·2355
$V2R_2$	77·0900	111·6950	94·3925	80·8475±6·7980
$W1R_{22}$	56·8245	50·0832	53·4539	46·1848±3·0081
Height				
E1	13·4000	19·0347	16·2174	44·5332±4·8195
E2	19·0898	21·7900	20·4399	28·9251±5·2311
$V1F_2$	68·6203	92·7091	80·6647	72·5561±9·6720
$V2F_2$	52·5125	64·5425	58·5275	66·3487±6·7454
$W1F_{22}$	28·7753	30·4814	29·6284	43·6310±5·9697
$V1A_2$	61·6988	45·5600	53·6294	48·1345±9·8996
$V2A_2$	63·5650	92·4350	78·0000	66·3487±6·7454
$W1A_{22}$	11·2424	16·1868	13·7146	19·2094±6·5783
$V1R_2$	60·4842	30·7604	45·6223	50·7406±7·1796
$V2R_2$	80·4250	101·2450	90·8350	66·3487±6·7454
$W1R_{22}$	11·3737	9·4379	10·4058	21·8155±2·9848

accurate information can be obtained by methods allowing the simultaneous utilisation of all the possible second degree statistics and this has been accomplished by the methods first discussed by Mather (1949*a* and *b*). An added advantage is that the methods depend mainly on the use of 3rd generation statistics based on large numbers of progeny and place no weight on statistics derived solely from small numbers of parents. At the same time they allow the detection of dominance and interaction as disturbing influences on the estimation and interpretation of the components of variation.

As previously noted we can best test the hypotheses outlined by comparing the total genetic variation obtained under each mating system. The first analyses assume the absence of dominance and reference to table 1 indicates that under these conditions only the 1st rank statistics of the *A* generation are inflated by D′ items. Since progenies derive from a common parental population the total potential genetic variation (G) must be a constant for all mating systems. Consequently the proportion of G appearing in the *A* 1st rank statistics can be estimated relative to the proportions appearing in all the remaining statistics. This would be most directly accomplished by allowing the coefficient of G for the *A* 1st rank statistics (a_A) to take its own value while allotting to the remaining statistics the coefficients fixed by hypothesis. But in point of fact the estimation

TABLE 6

Values of components and estimated coefficients of V_{1A_3} *and* $W_{1R_{23}}$ (i.e. a_A)

	Flowering time		Height	
	1952	1953	1952	1553
G	37·81±2·17 (31·30±2·46)	184·74±12·03	31·09±2·59 (28·31±1·78)	87·26±11·94
G_1	24·05±2·39 (22·65±2·59)	112·93±13·26	19·77±2·85 (19·17±1·87)	38·24±13·16
E_1	18·38±0·88 (18·48±1·33)	34·66± 4·86	10·50±1·04 (12·19±0·97)	44·53± 4·82
E_2	7·69±0·95 (9·08±1·04)	34·15± 5·27	6·07±1·13 (6·67±0·75)	28·93± 5·23
G_1/G	*0·64±0·07 (0·72±0·09)*	*0·61±0·08 (±0·01)*	*0·64±0·10 (0·68±0·07)*	*0·44±0·10 (±0·10)*
a_A	*0·32±0·035 (0·36±0·045)*	*0·31±0·038 (±0·005)*	*0·32±0·048 (0·34±0·035)*	*0·22±0·050 (±0·050)*
C.F. $a_F = 0·5$; $a_R = 0·25$				

was achieved by retaining the theoretical a_A value of $\frac{1}{2}$ and allowing G itself to assume its own value. The formulæ for V_{1A3} and W_{1A23} now become $\frac{1}{2}G_1+E_2$ and $\frac{1}{2}G_1$ respectively, G_1 thus being a distinct variable in the least squares equations. a_A is then obtained as $\frac{1}{2}\left(\frac{G_1}{G}\right)$ where G is estimated from the remaining statistics jointly. Since in this type of analysis factors affecting the value of G are indistinguishable from those affecting the coefficients of G (see also Bateman and Mather, 1951), this final estimate of a_A is the same whether G or its coefficient is allowed to vary.

The least squares estimates of the four components G, G_1, E_1 and E_2, calculated from the means of blocks, for both characters and both experiments are given in table 6. Values of the components can also be found for blocks separately and the values may be substituted

into the formulæ to give expectations for the statistics for each block and the block means. The deviations of observed from expected can then be analysed in the way described by Mather and Vines. The final results of the analyses are given in table 7.

The strict error variation which is obtained from differences between duplicate estimates of the same statistic (one from each block) has been partitioned into the two heterogeneity items shown; but since in no instance are the two items sufficiently different to merit separate consideration the appropriate duplicate M.S. is taken as the basic error in all cases. The residual disturbances item measures

TABLE 7

Analysis of variation for height and flowering time

Item	1952				1953		
	N	SS	MS	P	SS	MS	P
Flowering time							
Residual interaction .	7	979·59	139·94	<0·001	1480·24	211·46	>0·2
Heterogeneity of duplicates—							
Residual interaction	7	33·35	4·76	...	1115·72	159·39	...
Components .	4	19·69	4·92	...	512·39	128·10	...
Total . .	18	1032·63	...	...	3108·35	...	...
Height							
Residual interaction .	7	120·44	17·2061	0·2-0·1	4297·75	613·96	0·05-0·01
Heterogeneity of duplicates—							
Residual interaction	7	51·46	7·35	...	867·29	123·90	...
Components .	4	23·84	5·96	...	735·74	183·94	...
Total . .	18	195·74	...	...	5900·77	...	...

the departure of the observed mean value of each statistic from that expected under the conditions of no dominance, etc., outlined, and will thus include differences ascribable to dominance, genic interaction and linkage. It will be observed that only for flowering time in 1952 and for height in 1953 is there good evidence of one or more of these agencies affecting the expression of the character. In the other two cases, therefore, we can assume that the value of a_A derived from the ratio $\frac{G_1}{G}$ will be affected only by the degree of phenotypic correlation achieved in the A parents and the extent to which genotypic misclassification has resulted from environmental fluctuations.

The ratios $\frac{G_1}{G}$ and the values of a_A estimated from them are given in table 6. In all cases the standard errors attached to the components and to the ratios have been calculated using as basic error the duplicate M.S. weighted by means of the appropriate elements of the c-matrix used in the estimation of the components.

Consider first the results for 1952. In flowering time and height the values for a_A, in both cases 0·323 are higher than the theoretical a_R value of 0·25, but only in the case of flowering time is the error sufficiently low for significance to be approached (P = 0·1-0·05). The values are, in both instances, significantly lower than that expected under complete genotypic correlation. Thus, compared with the response achieved in height with a relatively weak parental correlation, the response obtained in flowering time is disappointing. Doubtless this can in large measure be related to the presence of dominance, interaction or linkage demonstrable in the flowering time analyses but in order to observe whether any individual statistic was having an undue effect in the estimation of $\frac{G_1}{G}$ ratios, standard errors have been calculated for the expected values of the statistics. Both the expected values and the standard errors are given in table 4.

It becomes apparent that two statistics show extreme deviation from expected. These are V_{2A3} and the direct estimate of E_1. Of course, in so far as V_{2A3} comprises a term for E_1 any sharp deviation in the former must affect the estimation of the latter. But why V_{2A3} should be almost double the values observed for the other 2nd rank variances is not immediately clear. Reference to table 1 shows that the genetic variance of the 2nd rank statistic is inflated for A generations relative to F and R generations by reinforcing dominance particularly where the numbers of genes involved are small. But this could hardly account for the large differences observed unless certain forms of genic interaction yield additional inflationary components of variation for this statistic under a system of assortative mating. Until further information is available on this point it would seem desirable to confine the estimation of G to the statistics available from the F and R generations.

Accordingly, for flowering time in 1952, the four components have been re-estimated, omitting V_{2A3} and also the direct estimate of E_1. Although for the 1952 height data deviations in these two statistics are not nearly so marked, V_{2A3} is still suspiciously high, and to keep the analyses comparable the components have been recalculated on the same basis as for flowering time. The new values for components and ratios together with standard errors are given in brackets in table 6. In both flowering time and height a_A values are now significantly higher than the theoretical a_R value (P = 0·05-0·02 in both cases).

Both characters therefore show increased genetic variability

relative to that obtained under random mating. In neither case is the increase a large one ($a_A = 0{\cdot}34$ as opposed to an expected 0·5) but for height this must be expected since the correlation between parents was by no means great ($r = 0{\cdot}38$), and as we shall see later the apparently small increase demonstrated for flowering time can be related to the greater complexity of its genetical control.

Turning to the 1953 results it can be seen that only in the case of flowering time is there any indication that a_A is greater than a_R. The standard errors, shown out of brackets, calculated by the methods employed for the 1952 analyses are too high to give any significance to this difference ($t_{11} = 1{\cdot}58$ and $P = 0{\cdot}2\text{-}0{\cdot}1$). The individual block values of the components demonstrate, however, that the values of G and G_1 vary but little from block to block. Standard errors, calculated using the duplicate M.S. as the basic error, are in fact inflated by large variation in E_1. Since we are concerned solely with variation in G and G_1 it is permissible to calculate the ratio G/G_1 for blocks separately and then estimate the standard error of the mean as $\sqrt{\frac{1}{2}\Sigma dev^2}$. For the two blocks the ratios are $\frac{115{\cdot}73}{187{\cdot}03} = 0{\cdot}62$ and $\frac{110{\cdot}13}{182{\cdot}45} = 0{\cdot}60$, and the standard error of the mean 0·61 is then 0·01. Based on this error the difference $a_A - a_R$ approaches significance although based on but 1 d.f. ($t = 11{\cdot}0_{(1)}$ and $P \doteq 0{\cdot}05$).

In height, as we should expect in so far as no significant phenotypic correlation could be demonstrated for the parents, the estimated A coefficient of G is in good agreement with that fixed for a random mating series (*i.e.* $a_A = a_R$).

Compared with the 1952 results the a_A value for flowering time is disappointingly low when it is remembered that in this experiment the genes controlling this character appear to be wholly additive in effect. The smaller number of plants used in the formation of a parental pool (40 as opposed to 100) must, however, involve a considerably higher sampling error as a limiting feature in the response expected.

So far we have not allowed for the effects of dominance or other genetic disturbances but the analyses suggest that, for the 1952 flowering time and the 1953 height, such agencies are affecting the expression of the character. For the 1952 flowering time data, therefore, it would be useful to try and establish the nature of these agencies and at the same time attempt some assessment of their effects on the assortative mating system.

(ii) *Dominance analysis of the* 1952 *flowering time data*

The data obtained for the F and R generations may be subjected to analysis allowing explicitly for, and hence estimating, the effects of dominance. Since formulation of similar expectations for the A statistics involve arbitrary assumptions as to the direction and

magnitude of dominance, these have been omitted from the analyses. The method of analysis is precisely that of Mather and Vines and so only final results are considered. The values of the four components D, H, E_1 and E_2 for flowering time in 1952 are given in table 8, together with the final analysis : the standard errors of the components have been calculated in the usual manner, using the duplicate M.S. as the basic error since the heterogeneity items are of the same order and can thus be pooled as a single error item.

The analyses give good evidence of significant dominance ($P = 0{\cdot}01$-$0{\cdot}001$) and some indication that $H > D$ ($P = 0{\cdot}1$-$0{\cdot}05$). It will also be noted that the item for residual interaction is significantly high, indicating disturbances caused by genic interaction and/or linkage. Now Hayman (unpub.) claims to have shown that where

TABLE 8

Analysis of variation in flowering time, 1952 (F *and* R *statistics*)

Components of variation	
D	25·1754± 4·0729
H	52·4392±14·7655
E_1	9·9711± 1·4781
E_2	7·7678± 1·1966

Analysis of residual variation

Item	SS	N	MS	P
Total SS	304·5552	12	...	...
Residual interaction	256·3698	4	64·0925	0·01-0·001
Heterogeneity of—				
Residual interaction	29·3644	4	7·3411	6·0232 (8)
Components	18·8210	4	4·7053	...

the numbers of interacting genes are small and the generation number low, genic interaction inflates H relative to D. Hence the high H value combined with the significant residual interaction item suggests that in addition to dominance there is a certain amount of genic interaction exhibited by the data. Since Mather and Vines have found linkage between the genes controlling flowering time in this cross, the high M.S. for residual interaction may also in part be attributable to disturbances caused by linkage.

The analyses indicate, therefore, that in the 1952 experiment the genetic control of flowering time was much more complex than that of height and the estimation of a_A for flowering time assuming simple additivity is not strictly valid. Obtaining an unbiased estimate of a_A under these conditions of high dominance, and possibly genic interaction, is not, however, likely to prove easy since some knowledge

of the properties of individual gene pairs is first required. In the present data the H/D ratio indicates at least full dominance of the majority of the genes concerned and the behaviour of the F_1 relative to the parents suggests that the dominance is preponderantly in the direction of early flowering. Neglecting any genic interaction we may argue that this most nearly approaches the first of the hypothetical situations earlier considered, where dominance was assumed to be complete and unidirectional for all genes. Reference to table 1 demonstrates that where n is large the proportions of the total genetic variations expected for V_{1A3} and W_{1A23} under these conditions approximate to $\frac{23}{48}$ G and $\frac{1}{2}$G respectively, while G expectations for the F and R statistics can be correspondingly modified to allow for H. Then if V_{2A3} and E_1 are again omitted and the $\frac{1}{48}$ difference between the 1st rank A statistics ignored, a new inverse matrix derived as for the previous 1952 analysis may be employed to give fresh values for the four components and so enable the recalculation of the ratio G_1/G.

The value of a_A calculated from this ratio is $0{\cdot}43 \pm 0{\cdot}017$. This is significantly higher than the theoretical coefficient of the V_{1A3} statistic of 0·31 ($t_9 = 7{\cdot}06$ and $P < 0{\cdot}001$), clearly indicating an increased genetic variance for the A families. It is, however, significantly lower than the theoretical a_A value taken as 0·48 (*i.e.* the V_{1A3} coefficient) ($t_{(9)} = 2{\cdot}91$ and $P = 0{\cdot}02$-$0{\cdot}01$), but it approximates nearer to theoretical expectation than when dominance is ignored; and when it is remembered that the phenotypic correlation between the parents was incomplete ($r = 0{\cdot}85$), this could be adjudged a satisfactory fit with expectation. At the same time it is likely that genic interaction, different dominance relationships of the genes and possibly linkage are additional variables affecting the estimation of this value for a_A.

This brief treatment of the data allowing specifically for dominance serves mainly to emphasise the difficulties involved in considering its effects on a system of assortative mating. Nevertheless the method employed could well provide a basis for further research into the matter and could also be modified to allow for the effects of genic interaction and linkage. But before this can proceed with any prospect of success there is need for more accurate methods of estimating the genetic components of variation.

5. DISCUSSION

Consider first the results for flowering time, the assorted character. In both experiments the analyses have enabled us to demonstrate that the proportion of the potential genetic variability detectable in an A generation (measured by a_A) is significantly greater than that

obtained under random mating (a_R). As we have seen (see Section 2), assuming the number of genes controlling the character to be large, this increased genetic variation cannot be ascribed to increased homozygosity as in the case of a selfed generation. The difference $a_A - a_R$, which for convenience we may denote by C, is founded rather upon non-random association of the genes in such a way that an excess of reinforcing genic combinations is obtained at the expense of the more balanced (opposing) combinations. Where the genes are additive in effect (and with no dominance) the difference may be related entirely to an excess of reinforcing hom/hom combinations, and C will be a measure of the D′ item of table 1. Where, however, the genetic control of the character is more complex, the increased variance will depend also on reinforcing hom/het and het/het combinations. Subject to the dominance relationships of the genes, C will then measure the combined effects of the D′, H′, B and B′ items of table 1. It will also measure other non-specified items if non-allelic interaction is operative. Measuring the contribution by each of the constituent items of C is, however, not likely to be an easy matter. In any case it would require a much more elaborate experimental structure than we have achieved in the present series. Nevertheless, the analyses have allowed the assessment of dominance and interaction in the data so that C can be interpreted accordingly.

In the 1953 experiment, where the analyses reveal simple additivity and no dominance for the genes concerned, we can assume that C depends entirely on reinforcing hom/hom combinations (D′), and continued perfect or strong assortative mating should fairly rapidly achieve fixation of genetic variability. But as Wright (l.c.) points out, the variability would be fixed for the most part in the free state as multiple reinforcement homozygotes and not as for selfing, where, in the absence of linkage equal proportions of free and homozygous potential variation (opposition homozygotes) are obtained. Furthermore, fixation will be achieved only within the assorted character, other characters, as is seen from a consideration of the height data, being affected only to the extent that they are correlated in expression with the assorted character. In the 1952 experiment where C for flowering time is also dependent on hom/het and het/het combinations (B, B′ and H′ items assuming only dominance), the advance to the fixation of free variability must still continue though at a slower rate. If we assume the genetic control to be more complex—involving genic interaction and linkage (especially in repulsion)—the fixation process is likely to be still further retarded but cannot be halted if the degree of genotypic correlation achieved by continued phenotypic assortment is sufficiently high.

Assorting on flowering time in the 1952 experiment also achieved a significant association of plants alike in height, and in the resultant generation it was possible to demonstrate significant C for this character. This effect may be deemed analogous to the correlated response

observed by Mather and Harrison (1948) as decreased fertility arising from selection for high and low chæta number in *Drosophila melanogaster*. That no such effect was observed in the 1953 experiment can, of course, be explained by the large sampling variation attendant upon the low flowering time/height correlation ($r \simeq 0{\cdot}45$ in both experiments). The 1952 results also illustrate that where genetic control is uncomplicated by dominance or interaction, a comparatively low order of phenotypic correlation (in this case $r = 0{\cdot}38$) is sufficient to evoke an observable response with regard to the distribution of heritable variation in succeeding generations.

The fact that we have been able to demonstrate significant changes in the distribution of heritable variation, although considering but a single generation, suggests that assortative mating may profitably have wider use as a technique in plant or animal breeding, especially where inbreeding methods are impracticable or undesirable. In experimental populations error variation tends largely to be under control so that a high degree of genotypic correlation should be attainable by perfect assortative mating. Although initially assortative mating does little to upset the homozygotic/heterozygotic balance, the heterozygotic state is maintained chiefly at the expense of the balanced or opposing homozygotic combinations, the free variability becoming gradually fixed within the population. Thus, since the breeder is usually concerned with extreme expression, selection based on assortative mating is not only useful as a practical means of securing the appearances which he requires (Nichols, 1947), but if continued should achieve the fixation of desired phenotypes without recourse to inbreeding. Moreover, where the genes controlling the character tend to be linked in opposition, this method of selection, by avoiding the premature homozygosis and fixation resulting from any system of inbreeding, should maximise the chances of securing and ultimately fixing desirable recombinants.

This could prove of particular value in the treatment of species showing marked inbreeding depression. Jinks (1954) has put forward the view that apparent overdominance appearing in maize and other species can be related largely to interaction between non-allelic genes. The rapid loss of vigour on inbreeding the hybrids can then be explained as close linkage in opposition of genes which show complete dominance and genic interaction. Should assortative mating be initiated among such hybrids and their derivatives, the frequencies of gene combinations giving extreme expressions would be maximised in certain of the resultant families. If the linkage relationships of the genes are such that favourable expression depends on heterozygotic (relational) balance, heterozygosis must initially be retained, but again the chances of procuring and retaining desirable recombinants would be maximised at each generation. Continued selection based on assortative mating should then increase the frequencies of such recombinants until they ultimately become fixed in the homozygous state. Furthermore, we

have seen that the extent to which other characters are affected depends on the strength of their correlation with the assorted character. Provided this correlation is not complete, it follows that this method of selection can also allow reassociation of genes controlling different characters. Thus favourable expression in characters other than that upon which selection is primarily based can be maintained or finally achieved.

Evidence deriving from recent selection experiments is manifestly in agreement with these views. For instance, the experiments of Mather and Harrison (1949) involved selection based on the assortative mating of extreme phenotypes in such a manner that inbreeding was reduced to a minimum. Although initially response to selection for high and low chæta number was accompanied by decreased fertility, they ultimately established true breeding lines having restored fertility and with chæta numbers considerably outside the range of variation observed in the original population. Admittedly, where the genetical situation is complicated, progress towards the fixation of desirable phenotypes is likely to be slow and improvement based on assortative mating would require long-term breeding programmes. In this respect further information as to the effect of assortative mating on a character showing high genic interaction may prove informative to breeders in general and those dealing with outbreeding crops in particular. Coupled with experiments designed to test the effects of assortative mating when combined with varying degrees of inbreeding in later generations, this information may reveal methods of dealing with material showing marked inbreeding depression in such a way that ultimately the commercial breeder need no longer depend upon the constant production of hybrids in the attainment of " hybrid vigour ".

Where the genotypic correlation achieved between parents is low, either by virtue of low phenotypic correlation (partial assortative mating) or because of high environmental variation, the degree of fixation will be smaller and the process being relatively slow will achieve importance only when measured on an evolutionary time scale. That a degree of assortative mating does occur as a natural phenomenon is evident, since before interbreeding can occur with any freedom prospective mates must occupy the same ecological niche and have their mating seasons coincidental. For instance, where a crossbreeding species exhibits variation in flowering time, interbreeding will be more frequent between plants flowering at the same time.

In a randomly breeding population the relative proportions of free and homozygous potential variation depends on the linkage relationships of the different genes. We have seen that where assortative mating is operative the proportion of free variability tends to be increased for the character in question at the expense of the homozygous potential variability. Populations affected must therefore

eventually reach a new equilibrium depending on the degree of genotypic correlation achieved by the mating system, such that with complete genotypic correlation the variability becomes finally fixed in the free state (see Wright, l.c.). Thus assortative mating can effect divergence within the population and if sufficiently strong can of itself form a basis for speciation. Furthermore, in crossbreeding species, the linked combinations of genes are likely to be relationally rather than internally balanced (Darlington and Mather, 1949), and "inbreeding depression" follows any departure from a crossbreeding system; but if we again explain relational balance as close linkage in opposition of genes showing interaction and/or dominance, assortative mating increases the frequency with which such linked combinations are broken and liberated to the action of selection as novel homozygotic combinations. In this way internal balance may be built up, and once established, would allow the development of inbreeding systems in species hitherto only able to survive as outbreeding units.

From the evolutionary standpoint, therefore, assortative mating can be both a force and a process. As a force it can of itself provide the impetus for divergence and speciation; as a process it provides a means whereby internal chromosomal balance may become established, and thus the first step to facultative inbreeding achieved, in hitherto obligatory outbreeding species.

6. SUMMARY

1. The possible consequences of a system of assortative (*i.e.* like to like) mating in quantitative inheritance have been considered for situations involving differing conditions of dominance. Experiments in *Nicotiana rustica* designed to compare the effects of assortative (*A*) relative to selfing (*F*) and random (*R*) mating systems have been analysed by means of the biometrical method of analysis developed by Mather (1949*a* and *b*), using flowering times as the continuously varying character.

2. The experiments demonstrate a significant increase in the genetic variability of *A* family means compared with that found under random mating. The extent of this increase depends (i) on the phenotypic correlation achieved between parents; (ii) on the degree to which genic expression is affected by environmental fluctuations; and (iii) on the complexity of the genetic control of the character. In no case was the genetic variation of *A* progenies so great as that found in the *F* generation.

3. If the numbers of genes involved is large, the increased variance of the *A* families cannot initially be attributed to any change in the homozygote/heterozygote ratio relative to that found under random mating. It depends, rather, on a re-association of the genes in such a way that an excess of combinations giving extreme expression is achieved at the expense of the more balanced combinations. Where

dominance is absent this must rapidly result in the homozygosis of reinforcing genic combinations and hence fixation of extreme expressions. With more complex genetic situations involving dominance, genic interaction and linkage, this process may be retarded but not necessarily averted.

4. The possible use of assortative mating as a technique in plant or animal breeding has been discussed. In evolution assortative mating is regarded as a force in the splitting of populations and hence speciation. It is also a process whereby the achievement of internal chromosomal balance may be accomplished. This is the initial and most important step towards the establishment of facultative inbreeding in hitherto obligate outbreeding species.

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THE GENETICS OF B CHROMOSOMES IN MAIZE

D. G. CATCHESIDE

Department of Microbiology, University of Birmingham

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B CHROMOSOMES in maize, and B-A interchange chromosomes with the B-chromosome centromere, are subject to non-disjunction at the second pollen grain division, with the result that the two male nuclei come to have unequal numbers of B chromosomes (Roman, 1947). These two unlike male nuclei have different probabilities of fertilising the egg in the embryo-sac, the one with the greater number of B chromosomes having the greater chance of fertilising the egg (Roman, 1948). As a consequence of these two factors, the progeny of maize plants with B chromosomes tend to have higher mean numbers of B chromosomes than do their parents (Randolph, 1941). Roman (1948) has observed differences in rate of mitotic non-disjunction in the pollen grains between the B^4 and B^9 interchanged chromosomes in TB-4*a* and TB-9*b* respectively.

Blackwood (1956) has also shown that when different maize plants are compared there is evidence that the probabilities of non-disjunction in the second pollen grain division and perhaps of preferential fertilisation differ from one strain to another. This is shown by the different progeny means and different progeny distributions of B-chromosome numbers obtained from different parent plants with the same numbers of B chromosomes. The problem is to make estimates of the magnitude of these two effects, so that they may be compared in different sets of data.

The most extensive data available concern crosses of the type 0×2 B, where only the pollen parent had B chromosomes. Suppose, in a plant with 2 B chromosomes the frequencies of cells (young pollen grains) with 0, 1 and 2 Bs are a, b and c respectively, immediately after the completion of meiosis. The sum, $b+2c$, may differ from unity as a result of loss or gain of B chromosomes through errors of movement or reproduction during meiosis, and presumably during preceding mitoses. Available data (Blackwood, 1956, table 3) show, amongst 550 postmeiotic cells, 39 with 0 B, 458 with 1 B and 53 with 2 B, the mean being 1·025 per cell. These values will be used for estimates of a, b and c.

If x is the probability that any B chromosome will undergo non-disjunction at the second pollen grain mitosis, and if the probabilities are independent for two B chromosomes present in the same pollen grain, the frequencies of B-chromosome distribution to the male cells of the pollen grains may be written down. However, before doing so, preliminary consideration must be given to the question of whether there is a preferred spindle pole to which the non-disjunctional B

chromatids pass. If there were, two non-disjunctional B chromosomes would always produce a 4 : 0 distribution. If, however, there were no preferred spindle pole, a 2 : 2 distribution would be produced in half of the cases of double non-disjunction. These alternatives, which respectively involve polarised and random segregation at the

TABLE 1

Distribution of B chromosomes to the male nuclei following the second pollen grain division

Distribution	Frequency	
	Polarised segregation	Random segregation
0+0	a	a
1+1	$b(1-x)$	$b(1-x)$
2+0	bx	bx
2+2	$c(1-x)^2$	$c(1-x)^2+\frac{1}{2}cx^2$
3+1	$2cx(1-x)$	$2cx(1-x)$
4+0	cx^2	$\frac{1}{2}cx^2$

second pollen grain division, lead to somewhat different expectations (table 1).

Preferential fertilisation can arise only from the third, fifth and sixth of these distributions. Suppose that the chance of fertilisation of the egg by the male nucleus with the larger number of B chromosomes is y, and that this chance is independent of the differential

TABLE 2

Proportions of progeny with different numbers of B chromosomes from 0×2 B crosses

Number of B chromosomes	Expectations	
	Polarised segregation	Random segregation
0	$a+x(1-y)(b+cx)$	$a+x(1-y)\left(b+\frac{cx}{2}\right)$
1	$(1-x)[b+2cx(1-y)]$	$(1-x)[b+2cx(1-y)]$
2	$bxy+c(1-x)^2$	$bxy+c-2cx+\frac{3cx^2}{2}$
3	$2cxy(1-x)$	$2cxy(1-x)$
4	cx^2y	$\frac{1}{2}cx^2y$

number of B chromosomes. Then the progeny of a 0×2 B cross will have plants with from none to four B chromosomes with the expectations given in table 2.

It will be noticed that unless x is greater than zero and y is greater than a half, the mean number of B chromosomes in the progeny cannot exceed the mean number in the parents.

It is now possible to obtain maximum likelihood estimates of x and y for any particular set of data. This is most simply done by

TABLE 3

Observed and expected frequencies of B chromosomes in the progeny of 0×2 B crosses. The expectations are computed from the estimates of non-disjunction and of differential fertilisation extracted from the data

(1) Data of Blackwood (1956)

Number of B's	Observed	Expected	
		Polarised segregation	Random segregation
0	16	16·2547	16·1388
1	8	6·9036	6·7390
2	50	60·2588	63·7480
3	1	1·2356	1·2017
4	16	6·3474	3·1726
χ^2 for 2 d.f.		16·6484	55·0994

(2) Data of Blackwood (1956)

Number of B's	Observed	Expected	
		Polarised segregation	Random segregation
0	27	27·4614	27·6473
1	48	46·4073	45·5429
2	66	69·5488	72·8175
3	5	5·5168	5·4206
4	8	5·0758	2·5717
χ^2 for 2 d.f.		1·9756	12·2766

(3) Data of Randolph (1941)

Number of B's	Observed	Expected	
		Polarised segregation	Random segregation
0	22	22·0022	16·7169
2	37	34·9530	42·0218
4	2	4·0448	2·2613
χ^2 for 1 d.f.		1·1536	2·3000

utilising Fisher's (1946) method of scores, and iteration after commencing with trial values. The parameters have been estimated for

three sets of 0×2 B data (table 3) with the results summarised in table 4. The values of a, b and c obtained by Blackwood (1956), given above, have been used for all three sets of data. It is an assumption that the same initial post-meiotic frequency distributions hold exactly for all, but it is probably not very far from being correct. It would be asking too much of the data to extract two more parameters from them.

It is clear that the frequency of non-disjunction of the B chromosomes is different for each set of data, the chance that any two could be alike being less than 0·01. The only uncertainty is that Randolph's data are theoretically compatible with a value of x less than unity; but even making an allowance for this, the probability that the value of x could be the same as in Blackwood (1) is not greater than 0·01.

The probabilities of differential fertilisation could be the same for all three sets of data, though the values for Blackwood (1) and Randolph have only a small probability (between 0·02 and 0·03)

TABLE 4

Estimates of genetic parameters of B chromosomes

Source of data	Mode of pollen grain segregation	Frequency of non-disjunction (x)	Frequency of preferential fertilisation (y)
Blackwood (1) .	Polarised	0·9113±0·0301	0·8716±0·0473
	Random	0·9135±0·0297	0·8671±0·0494
Blackwood (2) .	Polarised	0·6478±0·0419	0·8149±0·0499
	Random	0·6547±0·0419	0·8082±0·0513
Randolph . .	Polarised	1	0·6881±0·0662
	Random	1	0·7694±0·0648

of being the same. The differences between Blackwood's two sets of data are ascribable wholly to differences in the rates of non-disjunction during the second pollen grain division. If it is assumed, as may well be true, that there is no real variability in the frequency of differential fertilisation, a weighted mean value for all three sets of data may be calculated using the formula $\Sigma(y.I_y) \div \Sigma I_y$ where I_y is the information about y. The mean value is 0·8115±0·0307 for polarised segregation or 0·8227±0·0312 for random segregation.

If a comparison is made between the observed frequencies of progeny with different numbers of B chromosomes and the expected frequencies calculated using the estimates of x and y obtained above, better agreements are found with the expectations calculated on the assumption of polarised non-disjunction at the second pollen grain division (see table 3).

Roman (1948) has data for two different B-A interchange chromosomes, for whose behaviour the same two parameters may be estimated. In such interchanges the B^A chromosome, namely the one with the

B-chromosome centromere, may undergo non-disjunction at the second pollen grain division with a frequency x, giving two male nuclei respectively with 2 B^A and 0 B^A chromosomes. The hyperploid gamete preferentially fertilises the egg with a frequency y. Thus the progeny of a normal $\times B^A$ cross are of three types with respect to the B^A chromosomes and may be classified by suitable genetic tests (table 5). In the data quoted, a few progeny, obviously the result of heterofertilisation, have been omitted from the observations used for analysis; the 9 Type III individuals in the TB-9*b* data could also belong in this category. The estimates of x and y, also given in table 5, show, as Roman has reported, that the rates of non-disjunction at the second pollen grain division are distinctly and significantly different for B^{4a} and B^{9b}. However, contrary to Roman's implication (1948, p. 41, para. 2), the frequency of preferential fertilisation is

TABLE 5

Expectations and data for B^A interchange chromosomes in $O \times B^A$ crosses

(Data of Roman, 1948)

Type	Embryo constitution	Expected frequencies	Observed frequencies	
			TB-4*a*	TB-9*b*
I	0 B^A	$x(1-y)$	152	160
II	2 B^A	xy	382	327
III	1 B^A	$(1-x)$	124	9
		Totals .	658	469
Estimates of x			$0{\cdot}8116 \pm 0{\cdot}0152$	$0{\cdot}9819 \pm 0{\cdot}0019$
Estimates of y			$0{\cdot}7154 \pm 0{\cdot}0195$	$0{\cdot}6715 \pm 0{\cdot}0213$

not significantly greater in B^{4a} than in B^{9b}. The difference (0·0439) is only 1·519 times its standard error (0·0289), so that P is very nearly 0·13.

DISCUSSION

There are clear differences between the rates of non-disjunction of B chromosomes and of B-A interchange chromosomes at the second division in the pollen grain in different stocks of maize. However, there is no evidence to show whether the differences are due to innate differences between different B and B^A chromosomes or to general differences in the genotypes, residing chiefly in the A chromosomes. Roman (1950) has observed an absence of non-disjunction of B^4 chromosomes in unbalanced pollen grains which have a B^4 chromosome and a whole chromosome 4, but lack the 4^B chromosome. It was unknown whether the effect was due to the duplication of the distal part of the short arm of chromosome 4 or to the deficiency of the distal part of the B chromosome present in the 4^B chromosome.

Better agreement with experimental data is found if calculations of expected frequencies are made on the assumption of polarised non-disjunction at the second pollen grain division. If two non-disjunctional B chromosomes pass always to one spindle pole at P.G. II, it is possible that this division is specifically oriented so that the non-disjunctional B chromosomes, whether one or two, always pass to a specific spindle pole. It is possible that the axis of this division is in line with the axis of the first pollen grain division and that the B chromosomes pass to the outer spindle pole. Roman (1948) has previously suggested, as a possible cause of preferential fertilisation, a specific orientation of the deficient and hyperploid gametes within the pollen grain or at some time prior to fertilisation. The polarised segregation would lead to one of the male nuclei having a positional advantage over the other in respect to union with the egg, and it is particularly attractive to have one hypothesis uniting the two effects.

Presumably the non-disjunction is an interaction between the B chromosomes and a gradient of organisation within the pollen grain. The intensity of the effect may be expected to vary accordingly as the genotype controls the gradient; it is possible, also, that different B chromosomes might respond differently to the same gradient.

It is less easy to see how preferential fertilisation could be caused to vary. This might depend upon the degree to which the specific orientation of the two male nuclei, established in the pollen grain, was preserved or disturbed in the germination of the grain and during the growth of the pollen tube. However, the evidence for variation in the attribute of preferential fertilisation is not very strong.

In the estimates made above, preferential fertilisation is shown by values of y which are significantly greater than 0·5. This effect is of course manifested only in respect of those pollen grains whose male nuclei differ in their content of B chromosomes. If a coefficient of preferential fertilisation were needed, perhaps the most convenient would be $(2y-1)$, the difference between y and $(1-y)$. The variance of this coefficient would be twice the variance of y. The values for the three sets of data are 0·7432 for Blackwood's (1), 0·6298 for Blackwood's (2) and 0·3762 for Randolph's.

SUMMARY

Estimates have been made of the rates of non-disjunction of B chromosomes in maize at the second pollen grain division and of preferential fertilisation of the egg by the male gamete with the larger number of chromosomes. The rates of non-disjunction vary between different stocks, but there is no definite evidence that preferential fertilisation varies significantly. There is a strong probability that the second pollen grain division is polarised and a possibility that the resulting arrangement of the two male gametes determines preferential fertilisation.

This work was carried out in the Department of Genetics in the University of Adelaide.

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THE INHERITANCE OF B CHROMOSOMES IN *ZEA MAYS*

MARGARET BLACKWOOD
Botany School, University of Melbourne

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1. INTRODUCTION

Some stocks of maize carry supernumerary or B chromosomes, whose morphology and mode of inheritance differ from that of the normal or A chromosomes. The B chromosomes tend to be unequally distributed to the gametes, so that progeny plants appear with numbers of B chromosomes both larger and smaller than the mean number present in the parents. The mean number and the distribution of B chromosomes to the progeny is different according to whether they are contributed through male or female gametes. When the B chromosomes are carried by the male parent only, the number of plants with even numbers of B chromosomes (including zero as an even number) may exceed those with odd numbers.

There has been some suspicion that B chromosomes may be lost from a plant, or from some cells of it, so that there must be some compensatory mechanism to maintain the frequencies in the population. Darlington and Upcott (1941) considered that the B chromosomes confer a selective advantage upon the plants possessing them, to compensate for the mitotic losses which they observed. Randolph (1941) and Roman (1948) have suggested that the presence of B chromosomes is disadvantageous to the plants. They found non-disjunction of B's at the second pollen-grain division and preferential fertilisation of the egg by the gamete carrying the B's. These mechanisms together tend to ensure the retention and possible increase of B chromosomes in the population.

This investigation was undertaken to assess the effects of non-disjunction and preferential fertilisation upon the maintenance of B's in the population. Particular attention was devoted to the detection of genetic control of these behaviours.

2. MATERIALS AND METHODS

All the stocks used were provided by Dr D. G. Catcheside, then at Cambridge, who had received the foundation seed containing B chromosomes from Dr Herschel Roman, University of Washington, Seattle, Washington, U.S.A. The B stocks were crossed to stocks from other sources.

All material was fixed in acetic-alcohol used as a 1 to 3 mixture. After two days' fixation it was stored in a refrigerator at about 4° C. Squash preparations of roots and pollen mother cells were stained with iron-aceto-carmine and mounted in " Euparal ".

The B chromosome numbers of plants used in crosses were determined in the root tips of seedlings and were later checked in pollen mother cells. The progeny B chromosome numbers were counted only in root tips.

The work was carried out at the Botany School, Cambridge, and a field plot at the Rockefeller Experimental Station there was made available for planting during 1949 and 1950.

3. EXPERIMENTAL

Non-disjunction of the B chromosomes was found to occur at anaphase I of meiosis and also at the second division of the pollen grain nucleus. Observations of the distributions of B's at various stages in the development of pollen mother cells and pollen grains and in the progeny of plants with known B numbers, were made in order to estimate the rates of non-disjunction and of preferential fertilisation.

These observations were made in plants with 1, 2 and 3 B chromosomes and in the progeny from crosses of these plants with plants with no B chromosomes. All the B's observed in these stocks were of the standard type described by McClintock (1933), constant in size and appearance, club-shaped at metaphase of mitosis, about two-thirds of the length of the smallest A chromosome in maize, and characterised by the large amount of heterochromatin at the distal end.

(i) *Somatic aberrations*

There is no significant loss of the B's by fragmentation and irregular movement during mitosis in the root tip. Of 355 root tips examined, 3 only (less than 1 per cent.), showed different B chromosome numbers in cells of the same root, and then the major occurrence of variation was in one plant, suggesting some genetic control of this variation. The infrequent occurrence of such variation excluded it as an important factor in the inheritance of the B's. In 62 plants, whose roots and, later, pollen mother cells were examined, only two showed a change —*i.e.* in 3·2 per cent. of the plants. One showed a loss of one B and the other a gain of one B. Evidently, irregularities in B chromosome behaviour are infrequent in somatic cells so that there is little change in the number of B's between the embryo and the adult. Any irregularities seem to occur in the seedling stage rather than at later stages of development.

(ii) *Meiotic behaviour*

During meiosis the B's lag behind the A chromosomes on the spindle, but no loss of B's was observed as a result. However, precocious division of the lagging B's at anaphase I was found in the pollen mother cells with 1 and 2 B chromosomes. When followed by regular division at anaphase II, this resulted in a doubling of the number of B's present in the tetrad cells.

In 25 per cent. of the cells showing lagging B's at anaphase I in the 1 B plant, the single B had divided into two (fig. 1). From such a cell, assuming regular division at anaphase II, each tetrad cell

TABLE 1

Distributions and disjunction of B's at anaphase I and anaphase II in plants with 1, 2 and 3 B chromosomes

	1 B				2 B						3 B			
Anaphase I . .	0-1	1-1	0-2	Total cells	1-1	0-2	1-2	2-2	Total cells	Per cent. non-disjunction	1-2	0-3	Total cells	Per cent. non-disjunction
	32	7	3	42	86	13	2	2	103	9·8	67	11	78	12·0
Per cent. frequency .	76·2	16·6	7·2	...	84·2	12·6	1·6	1·6	...	...	85·9	14·1	...	...
Anaphase II . .	0-0 and 1-1	1-1 and 1-1	0-0 and 2-2	Total cells	1-1 and 1-1	0-0 and 2-2	1-1 and 2-2	2-2 and 2-2	Total cells		1-1 and 2-2	0-0 and 3-3	Total cells	
	41	6	2	49	46	8	1	0	55	0·0	29	4	33	0·0
Per cent. frequency .	83·7	12·3	4·0	...	83·6	14·5	1·9	0·0	...	...	87·8	12·2	...	...

would contain one B instead of half having one B and half none. If the two products of this precocious division both moved to the one pole, tetrad cells could be produced with 2 B's in half of them and no B's in the other half (fig. 2). If, in addition, chromatid non-disjunction occurred at anaphase II, then it would be possible for one cell in some tetrads to have 4 B's. Three cells at anaphase I showed a 0-2 distribution of the B's and two cells at anaphase II showed a 2-2 and 0-0 distribution (table 1). Both must have arisen from precocious division of the single B at anaphase I.

Precocious division was observed in 3·2 per cent. of the cells examined with 2 B chromosomes. The two B's had each divided along the axis of the spindle while lying apart from and between the two groups of A chromosomes at mid-anaphase. They, presumably, would form four single B's and their distribution at random in the

TABLE 2

Associations of B chromosomes during meiosis from pachytene to metaphase I in plants with 1, 2 and 3 B chromosomes

Stage	1 B			2 B			3 B			
	with A	I	Total cells	2 I	II	Total cells	3 I	II and I	III	Total cells
Pachytene .	3	84	87	0	30	30	1	50	22	73
Diakinesis .	...	53	53	2	42	44	2	51	15	68
Metaphase I .	...	40	40	4	66	70	3	48	20	71

Note.—At pachytene, I means a single B chromosome and II means an association of two B chromosomes which have synapsed, while III represents three B chromosomes which have synapsed together. At diakinesis and metaphase, I means a univalent, while II means a bivalent, and III represents a trivalent association held together by chiasmata.

telophase I groups would be 2-2, 1-3 or 0-4. If only one of the two B's divides precociously, then the possible distribution would be 1-2 or 0-3. In the cells observed at anaphase I there were two with a distribution of 1-2 and two with 2-2 (table 1). These distributions could be the source of anaphase II cells with 2-2 and 1-1 or 2-2 and 2-2 distributions.

No pollen mother cells showing precocious division of B chromosomes were found in the 3 B plant (table 1), suggesting that such behaviour may be genetically controlled. Certainly, whether the B number present is odd or even is not the controlling factor.

Non-disjunction at anaphase I in plants with 2 B and 3 B chromosomes is shown in table 1. In the true sense, non-disjunction implies the movement of the two or more homologous chromosomes to the same pole at anaphase I, *i.e.* chromosome non-disjunction. The term

is also used to include chromatid non-disjunction, namely, the movement together to one pole of the two chromatids of each chromosome at anaphase of mitosis (Longley, 1927, Randolph, 1941, Darlington and Upcott, 1941).

In the 2 B plant at anaphase I, distributions of B's observed were 1-1, 0-2, 1-2 and 2-2, the last two being the result of precocious division. The 1-1 would have come from normal disjunction of a B bivalent or from two univalents passing to opposite poles. The 0-2 distribution would have come, presumably, from two univalents passing to one pole, but many more cells show this distribution than can be accounted for by such movement. At metaphase I only 5·6 per cent. of the pollen mother cells showed the 2 B chromosomes present as univalents (table 2). These would contribute equally to the B distributions of 1-1 and 0-2, so that only 2·8 per cent. of 0-2 would be expected as compared with the 12·6 per cent. observed. This difference, 9·8 per cent., is highly significant and may be attributed to chromosome non-disjunction of the B's in bivalents at anaphase I.

The B distributions at anaphase I in the 3 B plant provided evidence of the same kind of non-disjunction. Cells at anaphase I show 14·1 per cent. of 0-3 distribution of which only about one-seventh can be accounted for by cells which had 3 univalents at metaphase I. Non-disjunction of bivalents and perhaps trivalents must therefore have occurred in at least 12 per cent. of all pollen mother cells.

Non-disjunction of the B chromosomes at anaphase I is one of the factors contributing to progeny with numbers of B chromosomes both larger and smaller than those expected from the numbers present in the parents.

No chromatid non-disjunctions of B chromosomes were seen at anaphase II, and there are no significant differences between the distribution frequencies at anaphase I and anaphase II in plants respectively with 1, 2 and 3 B's (table 1).

(iii) *Pollen grain division I*

The first division of the pollen grain nucleus appears to be regular. No exceptional behaviour of the B's was noted, and chromatid non-disjunction at anaphase does not occur. The distributions of B's after the division (table 3) agree with the distributions observed at anaphase I and anaphase II, which have been calculated from the data recorded in table 1.

(iv) *B chromosome numbers in generative nuclei of pollen grains*

In plants with 2 B chromosomes, the proportions of daughter cells with 0, 1 and 2 B chromosomes are similar at the end of the meiotic divisions and after the first division in the pollen grain. Hence the observations made at these stages may be combined to give estimates

TABLE 3

Frequencies of B's in resulting cells after anaphase I and II, and the pollen grain divisions, with estimates of the proportions of pollen grains with particular numbers of B's in the generative nucleus in plants with 1, 2 and 3 B chromosomes

No. of B's resulting in cells after	1 B							2 B						3 B						
	B frequency				No. of resulting cells	Total no. of B's	Mean per cell	B frequency			No. of resulting cells	Total no. of B's	Mean per cell	B frequency				No. of resulting cells	Total no. of B's	Mean per cell
	0	1	2	3				0	1	2				0	1	2	3			
Anaphase I . .	35	46	3	...	84	**52**	*0·62*	13	174	19	206	**212**	*1·06*	11	67	67	11	156	**234**	*1·50*
Anaphase II . .	86	106	4	...	196	**114**	*0·58*	16	186	18	220	**222**	*1·01*	8	58	58	8	132	**198**	*1·50*
1st p.g. division .	127	154	14	...	288	**182**	*0·63*	10	98	16	124	**130**	*1·05*	6	20	24	4	54	**80**	*1·48*
2nd p.g. division .	15	17	7	1	40	**34**	*0·85*	...	...	...	...	...	...	...	...	...	...	...	...	...
Estimates of proportions of p.g.'s with particular B numbers in the generative nucleus	248	306	21	...	568	**348**	*0·61*	39	458	53	550	**564**	*1·02*	25	145	149	23	342	**512**	*1·49*

(table 3) of the proportions of pollen grains with 0, 1 and 2 B chromosomes in the generative nucleus. Similar agreement between the proportions at the different stages are shown by plants with 1 and 3 B chromosomes and may be used to provide estimates of B distributions in the immature pollen grains in these plants.

(v) *Pollen grain division II*

The second division of the pollen grain nucleus was observed only in pollen grains from the 1 B plant and then with extreme difficulty. To see the chromosome behaviour during this division, the cytoplasm must be extruded from the pollen grain wall and the abundant starch grains removed. As a result, the number of cells showing anaphase stages in which the chromosomes may be counted is remarkably few. The chromosomes at this stage are very small, and close together.

Evidence of chromatid non-disjunction was found amongst 20

TABLE 4

Distribution of B chromosomes at the anaphase stages of the first and second divisions of the pollen grain nucleus in a plant, No. 152, with 1 B chromosome

B distribution	0-0	1-1	2-2	0-2	1-3	No. of cells	Per cent. non-disjunction
B frequencies at 1st anaphase	60	77	7	...	...	144	*0·0*
B frequencies at 2nd anaphase at division of generative nucleus	5	8	1	5	1	20	*35·3*

anaphase stages in which the B numbers in the two groups of chromosomes could be counted. If the second division were regular, the only distributions expected at anaphase would be 0-0, 1-1 and 2-2. Five cells showed a 0-2 and one showed a 1-3 distribution (table 4). These two types could have appeared only as a result of chromatid non-disjunction, the first from division of a 1 B generative nucleus in which the two chromatids moved to the same pole, and the second from a generative nucleus with two B's, in which the chromatids of one B did not disjoin but moved together to one pole with one chromatid from the other B which divided regularly. Thus 6 B's out of 17 observed at the second division of the pollen grain nucleus showed chromatid non-disjunction. The rate of non-disjunction is 35·3 per cent., but the observations are consistent at the 5 per cent. probability level with rates between 14 and 62 per cent. The range of B numbers which can be transmitted to the progeny is thereby increased.

The apparent increase in the mean (0·85 as compared with 0·61)

at the second pollen grain division is probably due to some selection of the cells actually recorded, those with no B chromosomes tending to be omitted unless they are particularly clear.

(vi) *B chromosome distributions in the progeny*

The numbers of B chromosomes in progeny of crosses between plants with and without B chromosomes are both greater and smaller than those in the parents (table 5). The mean is higher and there is a greater range in the numbers of B's in progeny where they were inherited from the male parent than in the reciprocals. In crosses where the B chromosomes were carried only by the male parent a larger number of progeny plants have even numbers of B chromosomes

TABLE 5

Frequency distribution of progeny plants with different B chromosome numbers from reciprocal crosses between plants with and without B chromosomes

Parent B no.	B frequency								No. of plants	No. of B's	Per cent. gain	Per cent. loss	Mean	Variance of mean	Standard error of mean
	0	1	2	3	4	5	6	7							
1×0	12	**16**	...	...	...	...	...	...	28	16	*14·2*	...	0·57	0·0091	0·095
2×0	2	**31**	3	...	...	...	...	...	36	37	*2·0*	...	1·03	0·0039	0·063
3×0	2	**14**	12	1	...	...	...	...	29	41	...	*6·0*	1·41	0·0161	0·127
4×0	...	4	**12**	2	...	...	...	...	18	34	...	*5·5*	1·88	0·0178	0·133
5×0	...	...	5	**13**	7	...	...	...	25	77	*22·8*	...	3·08	0·0190	0·138
8×0	...	...	2	7	**16**	5	1	...	30	120	...	...	4·00	0·0089	0·094
9×0	...	...	...	3	**12**	11	1	1	28	125	...	*1·7*	4·46	0·0268	0·164
0×1	24	**36**	4	1	...	...	...	...	65	47	*37·5*	...	0·72	0·0065	0·081
0×2	9	18	**24**	2	3	...	...	...	56	84	*50·0*	...	1·50	0·0175	0·132
0×3	15	4	**21**	3	8	1	...	...	52	92	*18·0*	...	1·77	0·0434	0·208
0×4	5	2	**10**	3	7	1	...	...	28	64	*14·0*	...	2·28	0·0244	0·156

than odd numbers of B's, especially as the number of B's used in the cross increases. Finally, when the B number used in the cross is odd, there is a greater variance than when it is even, and as the B number increases there is an increase in the variance of the means when the odd and even numbered crosses are considered separately.

The first and second properties were observed by Longley (1927) for reciprocal crosses between plants with no B and 1 B chromosomes (table 6). Randolph (1941) observed the first three properties for many different reciprocal crosses between plants with and without B's and between plants in which both parents carried B's. His data also show clearly the increase in the variance with increase in the parental numbers of B's.

Comparisons of the distributions of B's in the progeny of crosses involving similar numbers of B's show significant differences between

the means in some cases, and in others, between the frequency distributions of B's. First, the progeny from reciprocal crosses between plants with no B's and plants with B's are statistically different. The means of Longley's two sets of frequencies in table 6 are not significantly different, but the distributions are ($\chi^2_2 = 19{\cdot}4$). Randolph's data in the same table show a significant difference between the means. Similarly, the means of reciprocal crosses between 0 and 2 B plants are significantly different, both for Randolph's crosses (1941) and those given in table 5. Next it was found that means of B frequency distributions in progenies from similar crosses of different origin could differ significantly. In the 1×0 and 0×1 crosses shown in table 6, the means of B and L are significantly different, as are also the means of B_1 and L_1.

These results suggested that the distribution of the B's to the

TABLE 6

The present, Longley's and Randolph's data, arranged for comparison, of frequency distributions of plants with different numbers of B chromosomes in the progenies from reciprocal crosses between plants with 0 B and plants with 1 B chromosomes

1×0	B frequency				No. of plants	No. of B's	Mean	Variance of mean	Standard error of mean
	0	1	2	3					
B.	12	16	...	...	28	16	*0·57*	0·0091	0·095
L.	109	58	3	...	170	64	*0·38*	0·0016	0·040
R.	46	19	...	...	65	19	*0·29*	0·0032	0·057
0×1									
B_1	24	36	4	1	65	47	*0·72*	0·0065	0·081
L_1	76	22	15	...	113	52	*0·46*	0·0046	0·068
R_1	32	13	5	2	52	29	*0·56*	0·0131	0·114

gametes, particularly to the male gametes, might be under genetic control. Crosses were made between seed parents with no B's and pollen parents with two B's, to answer particular problems :—

(1) Four unrelated plants known to have no B's were pollinated by a single plant with 2 B's (table 7, i-iv) to determine whether the genotype of the female parent would affect the range, frequencies, and distributions of the B's in the progeny ; and

(2) plants with no B chromosomes were pollinated by plants unrelated to one another and each with two B's (table 7, v-viii), to determine whether different male genotypes would give different ranges, frequencies and distributions of B's in the progeny.

The first four samples in table 7 do not differ significantly from one another, either in their means or frequencies of plants with different B numbers. Hence the female parent has no effect upon preferential fertilisation which, therefore, appears to be pre-determined by the pollen parent. These four progenies may be pooled to represent the performance of plant 136.

However, v differs significantly from i-iv combined, both in the mean number of B's in the progeny and in the frequencies of different numbers. This difference lies especially in the relatively greater number of plants with odd B numbers in v than in any of the other four families which all show a pattern of greater frequencies of plants

TABLE 7

Frequency distribution of progeny plants with different B chromosome numbers from crosses between plants with 0 B chromosomes as the seed parents, and plants with 2 B chromosomes as the pollen parents

Cross	Parent B no.	B frequency					No. of plants	No. of B's	Mean	Variance of mean	Standard error of mean
		0	1	2	3	4					
(i) 10×136 .	0×2	4	1	19	0	5	29	59	*2·03*	0·0455	0·213
(ii) 11×136 .	0×2	4	3	12	1	4	24	42	*1·92*	0·0649	0·255
(iii) 13×136 .	0×2	3	2	9	0	4	18	36	*2·00*	0·0980	0·313
(iv) 16×136 .	0×2	5	2	10	0	3	20	34	*1·70*	0·0847	0·291
Totals . .		16	8	50	1	16	**91**	171	*1·92*	0·0167	0·214
(v) 71/24×75/18 .	0×2	9	18	24	2	3	56	84	*1·50*	0·0175	0·132
(vi) 21×116 .	0×2	6	16	22	1	3	48	75	*1·56*	0·0194	0·139
(vii) 19×114 .	0×2	4	5	8	0	2	20	29	*1·45*	0·0434	0·208
(viii) 25×138 .	0×2	8	9	12	2	0	31	39	*1·25*	0·0171	0·131
Totals . .		27	48	66	5	8	**155**	227	*1·47*	0·0064	0·154
(ix) Randolph's .	0×2	22	0	37	0	2	**61**	82	*1·34*	0·0191	0·138

with even than with odd numbers of B's. However, this type of distribution is less exaggerated in i-iv than in Randolph's data (table 7, ix). The mean of ix differs significantly ($P<0{\cdot}01$) from that of i-iv but not from the mean of v. The frequencies of plants with different numbers of B chromosomes are very different in ix and v since there are relatively more plants with even numbers in Randolph's progeny. Indeed, v and ix differ very significantly ($\chi^2_2 = 24{\cdot}7$) in the distribution of frequencies of plants with different B numbers in the progeny.

The other progenies, vi, vii and viii resemble v more closely than i-iv or ix. There are, relatively, more plants with odd numbers of B's present and, in viii, with the absence of any 4 B plants, a more equal distribution of plants with 0, 1 and 2 B's and a low mean of

1·25, there seemed to be a shift towards a population with few or even no B chromosomes, even though it still shows a 25 per cent. gain over the expectation of 1 B chromosome per plant. However, the means and frequency distributions of vi-viii do not differ significantly from v nor between one another. It was therefore concluded that v-viii were sufficiently homogeneous to pool and compare with other data. Their mean does not differ from that of Randolph's family, but the frequency distributions are very different ($\chi^2_3 = 30{\cdot}1281$). The two groups i-iv and v-viii have significantly different means and frequency distributions.

Not only are unexpected B numbers found in progeny of a particular pollen parent, but different mean numbers of B's and dissimilar distribution frequencies occur in the progeny from similar crosses. These variations are dependent upon events which occur with variable frequency, in the development of the pollen grains and the events leading to fertilisation in the embryo sac. However, the seed parent is without effect upon the behaviour of the male gametes.

4. DISCUSSION

In these stocks, mitotic lapses of the B chromosomes have been found to be infrequent and the amount of loss or gain of B's during meiosis due to lagging and precocious division does not affect, significantly, the conclusions to be drawn from progeny analysis. Randolph (1941) considered such lapses to be insignificant in his material, yet Darlington and Upcott (1941) observed them frequently enough to suggest that the B's confer a compensating selective advantage to the plant. All these observations indicate that the frequency and extent of such aberrations vary between stocks. However, the different rates of precocious division found in cells with 1, 2 and 3 B chromosomes, namely 23, 3 and 0 per cent. is a reflection of the different frequencies of univalents in such cells.

Non-disjunction of the B chromosomes in maize and other plants has been reported as occurring at various stages during meiosis and at both the pollen grain divisions. In maize, Longley (1927) observed it at anaphase II, while Randolph (1941) and Roman (1947) both inferred from genetical results that it occurred at the second pollen grain division. In other plants, most cases of non-disjunction have been described as occurring at the first pollen grain division. Müntzing (1946) in *Secale*, Östergren (1947) in *Anthoxanthum* and Bosemark (1950) in *Festuca* all observed it at this stage and, in the last two examples, only on the male side. Håkansson described non-disjunction in the embryo sac of *Secale*. In *Secale*, in the pollen grain, directed non-disjunction takes place, so that the larger number of B's pass into the generative nucleus, increasing the number of B's per gamete.

The general effect of non-disjunction is to alter the distribution of B's in resultant cells, but not the total nor the mean number per cell. Daughter cells may have either smaller or larger numbers than

the parent and even rather than odd numbers of B chromosomes. The frequency of non-disjunction is reflected in the relative numbers of progeny with even and with odd numbers of B's, the rate of non-disjunction being inversely related to the proportion of plants with odd numbers.

In maize, non-disjunction occurs at two stages in the male parent during the reproductive phase. Chromosome non-disjunction occurs at anaphase I and chromatid non-disjunction at the second pollen grain division. They account for the wider range in B frequencies in the progeny than could be obtained from a single occurrence—*e.g.* from 0-4 for a 2 B plant. At anaphase I, the rate of chromosome non-disjunction in the 2 B and 3 B cells was of the order of 10 per cent. The rate of chromatid non-disjunction at the second pollen grain division, in the 1 B plant, was 35 per cent. Although similar observations were not made at pollen grain division II in plants with 2 and 3 B's, the progeny frequencies of B's indicated its occurrence at this stage. For instance, at the end of the first pollen grain division in the 2 B plant, the generative nucleus had either 0, 1 or 2 B chromosomes, but the progeny from the 0×2 cross included plants with 0, 1, 2, 3 and 4 B's. Plants with 3 and 4 B's must have originated from eggs fertilised by gametes with 3 and 4 B chromosomes, derived from non-disjunction of 2 B's at the second pollen grain division. Distributions of B's in progeny from crosses in which only the seed parent carried the B's, indicate that non-disjunction takes place at some stage in the development of the egg. The B distributions in the progeny from the crosses $2B\times0$ and $3B\times0$ are not significantly different from those at the first pollen grain division in the 2 B and 3 B plant (respectively, $\chi^2_2 = 0{\cdot}3824$ and $\chi^2_3 = 1{\cdot}4958$). The observations agree with the assumption that only chromosome non-disjunction occurs on the female side, at a rate equal to that in pollen mother cells. There is no evidence of chromatid non-disjunction at post meiotic division in the embryo sac.

In the progenies from crosses in which different pollen plants with the same number of B chromosomes were used as parents, the frequencies of the progeny plants with different B numbers showed differences sometimes in their mean and distributions, sometimes in their distributions alone.

A significant difference between the means of such progeny could be due to different rates of non-disjunction at the second pollen grain division with a given rate of preferential fertilisation, or to different rates of preferential fertilisation following a given rate of non-disjunction or to variation of both. Likewise variation in either factor may alter the distribution. Consequently, more searching analysis is needed to determine which factor is responsible for a particular effect.

Non-disjunction at the second pollen grain division leads to a relative increase in the proportion of gametes with even (including

zero) numbers of B's. The ratio of the number of progeny with even (including zero) to odd numbers of B chromosomes provides a measure of the rate of non-disjunction. The ratios are 0·9 for the first group (i-iv), 0·64 for the second group (v-viii) and 1·0 for Randolph's data.

It is clear that the rate of non-disjunction is inconstant for a particular chromosome number. It is not yet known whether the rate is constant for a particular plant throughout the period of pollen shedding. Also whether environment affects the rate is unknown. Certainly, different plants behave differently on the same day, but this could be a reflection either of different states of maturity or of innate genetic differences between plants.

Preferential fertilisation of eggs by male gametes with larger numbers of B's can only occur when non-disjunction at the second pollen grain division has taken place and will therefore lead, in the case of the 0×2 B cross, to a deviation from equality of the number of progeny with even as compared with zero B's. The proportion of heterogametic pollen grains in which the male gamete with the larger number of B's preferentially fertilises the egg is approximately the ratio of their differences to their sum of the number of progeny with even and zero numbers of B's. The ratio has the values of 0·61, 0·47 and 0·28 respectively, for the three groups of data. It would seem that the frequency of preferential fertilisation may also be variable and, if so, genetically controlled. However, such variability is unproven.

More precise estimates of these two factors need a more sophisticated treatment (Catcheside, 1956) because of the complex way in which the two interact.

If the presence of B's confers an advantage upon the plant, as Darlington and Upcott (1941) suggested, then with the mechanisms of non-disjunction and preferential fertilisation together giving rise to progeny plants with larger B numbers, an increase in the number of B's in the population during succeeding generations might be expected. There is no evidence for any such population gain. Rather it would seem that the presence of B's confers a selective disadvantage and that non-disjunction followed by preferential fertilisation ensures the retention of the B's in the population. It is not yet apparent what the disadvantage is, nor to what extent genetic control maintains the B chromosomes in the population.

5. SUMMARY

1. During mitosis irregularities in the behaviour of B chromosomes were infrequent. During meiosis, lagging and precocious division occurred with effects upon the distribution of B's to the progenies.

2. Chromosome non-disjunction of the B chromosomes was observed at anaphase I of meiosis in pollen mother cells and chromatid non-disjunction at the second division of the pollen grain nucleus.

3. Analysis of B chromosome distributions in the progeny showed that non-disjunction occurred at varying rates in different plants, even in unrelated ones with the same B number. This might be due (i) to differences in the A genotypes, the rate of non-disjunction and the stage at which it occurs being under genetic control, or (ii) to differences between the B chromosomes themselves.

4. The occurrence of preferential fertilisation was confirmed and, since this process, following upon non-disjunction, promotes the retention of B chromosomes in the population, and there is no population gain of B's, it is suggested that the B chromosomes confer a disadvantage upon the sporophyte.

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CHROMOSOME DISTRIBUTION AND SPONTANEOUS CHROMOSOME BREAKAGE IN *TRILLIUM GRANDIFLORUM*

ALFRED RUTISHAUSER
Institut für allgemeine Botanik Universität Zürich

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I. INTRODUCTION

In 1940 Darlington and La Cour showed that the metaphase chromosomes of *T. grandiflorum* and other *Trillium* species are composed of more and less strongly stained segments when they are exposed before fixation to a temperature near freezing point. The regions weakly stained with leuco-basic fuchsin were named " differential segments ". They correspond to the deeply stained chromocentres of resting nuclei, as Kurabayashi (1952*a*) was also able to show, and, therefore, they are identical to the heterochromatin of other organisms. Resende (1945) would prefer the name olisterochromatin for this chromatin type. In this paper, following a suggestion of Prof. Darlington, we use the abbreviation H-segments for heterochromatic segments.

Position and number of H-segments was constant within an individual in the experiments of Darlington and La Cour, but differences appeared when corresponding chromosomes of different test plants were compared with one another. Surprisingly, the partners of homologous chromosome pairs of the same plants often proved to be heteromorphic in so far as they could show variation in number and position of H-segments. Darlington and La Cour speak in such case of a hybrid chromosome complement.

The present work was originally undertaken with the intention of examining the question of the constancy of H-segments by crossbreeding experiments. These experiments were made possible by the fact that instead of F_1-plants the endosperms of seeds of cross-pollinated flowers were cytologically analysed. The method used was first applied to endosperms of lilies and Ranunculi (Rutishauser and Hunziker, 1950 ; Rutishauser, 1953).

In the course of investigations with species of the *Trillium* genus it was shown that the cytological analysis of the endosperm does not only give information about the constancy and behaviour of the heterochromatin in the crossbreeding experiments. It enables us further to follow the cytological consequences of " crossing-over " (Rutishauser, 1955*a*, *b*) and especially the phenomenon of spontaneous chromosome breakage more easily than was formerly possible. Some first results concerning the latter problem have already been published by Brock (1954, 1955).

2. MATERIALS AND METHODS

The samples were taken from three nurseries, C. Frikart Söhne, Stäfa/Zürich ; A. Vogt, Erlenbach/Zürich ; B. Ruys AG., Dedemsvaart, Holland. The samples from Switzerland were designated G_1, G_2 and the samples from Ruys, Holland, GH.

As I was concerned with obtaining a complete "starvation effect" in the chromosomes, experiments were first made with duration and temperature of the cold-treatment. A deep freezing chest of Therma AG., Schwanden, with a thermostat of Sauter AG., Basle, was used as a refrigerator. The tolerance of this freezing chest was $\pm 0{\cdot}2°$ C. Temperatures of $0{\cdot}3°$ to $1{\cdot}0°$ C. with a duration of 4 days proved optimal. In some cases plants were successfully kept in a temperature of $1{\cdot}3°$ C. for 5 days. Lower temperatures are unfavourable, because the chromosomes of the endosperm mitoses then become stickier, thereby making the investigation more difficult.

As a fixative for the roots, 2BD (La Cour, 1954) was used and for the endosperms either Carnoy or acetic alcohol. The description of the endosperm squash methods has already been given elsewhere (Rutishauser, 1956 and La Cour, 1954). Almost all endosperms were given the Feulgen treatment and post-stained with Orcein.

The staining capacity of the endosperm chromosomes decreases with the age of the seeds, but older endosperms were as a rule used in these investigations (fixation took place 5-6 weeks after pollination). The reason lies in the following peculiarities of endosperm development ; the mitoses in young endosperms are mostly completely synchronised, and, therefore, the number of endosperms with divisions is small. Later, after the formation of the cell wall, which takes place about 5 weeks after pollination, the mitoses are scattered throughout the whole tissue as in the root-tips. And so in favourable cases one can assume that each endosperm contains analysable mitoses, which is of great importance especially for genetic investigations.

The photographs were taken with a Zeiss Miflex camera and the drawings made with a Zeiss-Winkel camera Lucida.

3. CHROMOSOME COMPLEMENT OF PARENT PLANTS

In all 70 plants were investigated and number, position and length of heterochromatic and euchromatic regions in each metaphase chromosome ascertained, and in this way the chromosome complement of the test plants was collated. All investigations were carried out on mitoses of the root-tips. This process and the prolonged cold-treatment has a certain inherent disadvantage : the chromosomes are easily shortened and particularly the H-segments are less drawn out than in the preparations of Kurabayashi (1952*b*) and Haga and Kurabayashi (1954). But as the cytology of the endosperms of the same plant had to be investigated at the same time, it was not possible to use the ovules which were clearly more suitable for such experiments or to shorten the time of cold-treatment.

The process mentioned partly entails reservations being made in respect of the constancy of the H-segments. The large segments were completely constant in the root mitoses of an individual as far as their position and number is concerned. No losses or gains of heterochromatic or euchromatic substances could be established. In this regard my investigations confirm the results of Darlington and La Cour (1940) and Haga and Kurabayashi (1953, 1954). But it is different with the shorter segments. Here, differences can be pointed

out between identical chromosomes of different mitoses of the same plant. Such " unstable " heterochromatic regions lie mainly in the long arm of the D-chromosome (fig. 1) and also at the ends of the short arm of the D- and C-chromosomes (fig. 1).

Because of the difficulties the unstable segments present to analysis, I am limiting myself in this work mainly to the behaviour of the long constant regions. In fig. 1, however, all the chromosomes discovered have been marked in (the unstable regions are marked x). It can be seen that the number of different chromosome types, compared with

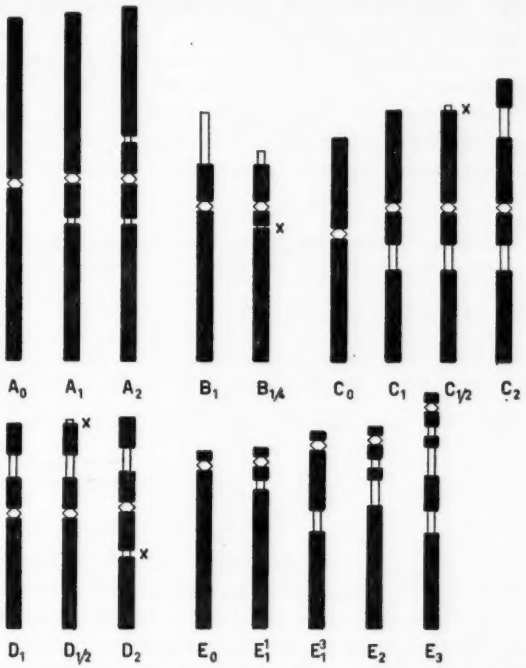

FIG. 1.—Chromosome types of *T. grandiflorum* (black : euchromatic segments, white : heterochromatic segments, X = unstable heterochromatic segments). ×2000.

the statements of Haga and Kurabayashi (1954) for *T. kamtschaticum*, is small. The greatest variability is shown by the E-chromosome. In all three samples four types of E-chromosomes could be distinguished (plate, figs. 1-4). They were named, E_0, E_1, E_2, E_3 according to the number of their H-segments. We shall go into the matter of the discovery that there are two different E_1-chromosomes later (p. 383). Constant heterochromatic blocks occur singly or in pairs in C-chromosomes also (plate, fig. 2). The B-chromosomes are distinguished by the length of the one terminal segment. They are named B_1 and $B_{1/4}$ (plate, fig. 3). The A- and D-chromosomes show least variation.

Only three types of A-chromosomes (A_0, A_1, A_2) can be distinguished with certainty. In many A-chromosomes, however, both short heterochromatic regions are not stable. All D-chromosomes possess a stable H-segment; all remaining H-segments are variable. In many cases, however, the H-segment in the long arm seems to be completely constant.

From table 1 it can be seen that one of the discovered chromosome types is always the most numerous. We call it the standard type, and a chromosome complement with only these types of chromosomes the standard complement. In the material examined it has the formula $A_0A_0B_1B_1C_1C_1D_1D_1E_2E_2$.

The relative frequency of deviating chromosome types varies from group to group and is at its greatest in the GH-sample (26·2 per cent.). Similar observations have been made by Haga and Kurabayashi (1954) with bigger plant-tests with *T. kamtschaticum*.

The 5 chromosome types of *T. grandiflorum* are affected in different

TABLE 1

Frequency of chromosome types in Trillium grandiflorum

Sample	No. of plants	A_0	A_1	A_2	B_1	$B_{1/4}$	C_0	C_1	C_2	E_1	E_2	E_3	Standard type	No. of aberrant chromosomes	
														total	per cent.
G_1	42	84	...	...	76	8	2	78	4	1	82	1	320	16	4·8
G_2	8	16	...	...	15	1	...	16	...	6	7	3	54	10	15·6
GH	20	24	15	1	38	2	...	33	7	5	23	12	118	42	26·2
Total	70	124	15	1	129	11	2	127	11	12	112	16	492	68	12·1

degrees by variability. In the G_1-sample it is the B- and C-chromosome, and in the G_2-sample the E-chromosomes which vary most widely. The GH-sample shows variability in all chromosomes. In the average the E-chromosomes seem to be the most unstable, 20 per cent. of all E-chromosomes deviate from the standard type.

Darlington and La Cour discovered (1940) that even the homologous chromosomes of *T. grandiflorum* can vary in heterochromatin content. Their observations were confirmed by Haga and Kurabayashi (1954) and could also be confirmed in my experiments (table 2). As is to be expected the frequency of heteromorphic chromosome pairs depends on the degree of variability of the different chromosomes (table 1).

Surprisingly the number of plants with aberrant homomorphic chromosomes is relatively small. The combinations E_1E_1 and E_3E_3 appear only twice each, C_2C_2 only once. The $B_{1/4}$-chromosome does not appear at all in a homozygous condition. This situation is analogous with that in *Campanula persica* (Darlington and Gairdner,

1937) where only " basic types " are homozygous. Like the interchanges of *Campanula* the aberrant chromosomes of *Trillium* float in heterozygous combinations.

To obtain more detailed information about the relationship between heterochromatic and euchromatic regions, measurements were made of twenty chromosomes of each different type. They show that the total length of the euchromatic regions always remains about the same. Only the heterochromatin is variable.

Besides the usual chromosomes many plants in all three experimental groups possess fragment chromosomes as well. A report on their morphology and genetics was given in an earlier work (Rutishauser, 1956).

TABLE 2

Frequency of heteromorphic and homomorphic chromosome pairs

Sample	No. of plants	A		B		C		E		Total		
		hom.	het.	hom.	het.	hom.	het.	hom.	het.	hom.	het.	per cent.
G_1	42	42	...	34	8	36	6	40	2	152	16	10
G_2	8	8	...	7	1	8	...	2	6	25	7	22
GH	20	9	11	18	2	13	7	8	12	48	32	40
Total	70	59	11	59	11	57*	13	50†	20	225	55	20

* 56 C_1C_1-pairs and 1 C_2C_2-pair.
† 46 E_2E_2-, 2 E_1E_1- and 2 E_2E_3-pairs.

4. DEVELOPMENT OF THE EMBRYO-SAC AND THE ENDOSPERM

The embryo-sac development of *T. grandiflorum* was investigated by Ernst (1902) and it was found that here was a case of the *Allium* type.* The same is true according to investigations of Coulter and Chamberlain (1915) for *T. recurvum*, according to Heatley (1916) for *T. cernuum*, and according to Spangler (1925) for *T. sessile*. The *Allium* type of embryo-sac development is characterised by the fact that a cell wall is formed only after the first meiotic division of the EMC (fig. 5). Two dyads are formed : the upper one degenerates. The nucleus of the lower one undergoes three further divisions, so that an 8-nuclear ES is built up. The first of these three divisions corresponds to the second meiotic division. The statement that the two polar nuclei derive from the two tetrad nuclei which develop in connection with the second meiotic division, is important for our cytological problem. As has been shown earlier (Rutishauser, 1955*a*, *b*) it thus becomes possible to carry out " tetrad analysis " by investigation of endosperm mitoses.

* The accounts of Jeffrey and Haertl (1939) regarding the embryo-sac development of *T. grandiflorum* was proved to be erroneous by Blain and Howe (*cit.* from Maheshwari 1947).

The course of endosperm development in *T. grandiflorum* was investigated with endosperms which were prepared *in toto* from seeds. Fig. 2 gives information about the sequence of the nuclear divisions in the two chambers, the chalazale and the micropylar. From the youngest observed stage on (8-nuclear endosperm) the development proceeds to the 64-nuclei stage in such a way that the nuclei of the micropylar chamber divide first, and then the nuclei of the chalazale chamber follow. The development of the micropylar chamber seldom is further in advance : only once was an endosperm observed whose micropylar region had 32 prophase nuclei, while the chalazale was still in the 16-nuclear stage.

From these observations it follows that the division impulse affects the micropylar chamber first, then spreads to the chalazal chamber. Contrary to the suppositions of Swamy (1948) about the endosperm development of *T. undulatum* both chambers appear to be equivalent as far as the number of the nuclei is concerned. On the other hand differences of another kind appear quite early. The chalazal chamber is lower than the micropylar one even in the earliest observed stage, the nuclei therefore lie closer together. This difference is strengthened

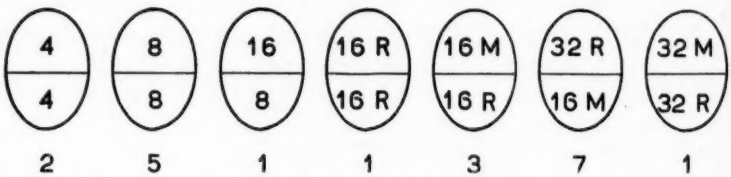

FIG. 2.—Endosperm development of *T. grandiflorum*. M = mitoses, R = resting nuclei. Below, number of observations.

with the growth of the endosperm. The most striking difference concerns the reaction to leuco-basic fuchsin : the nuclei of the chalazal chamber are always much more deeply stained than those of the micropylar ones.

The abnormal behaviour of the chromosomes under the influence of cold presumably stands in close relation to this property of the chalazal endosperm nuclei. In the chromosomes of the chalazal endosperm the starvation effect is much less marked than in the chromosomes of the micropylar nuclei. The H-segments are as a rule more darkly stained and in many chalazal mitoses the differential effect is completely absent.

These observations are of great interest because they possibly hold the key to the understanding of further abnormal processes which are mostly connected with the chalazal region of the endosperm. As Swamy (1948) has already stated for *T. undulatum* and a whole series of embryologists before him for plants with helobial development of the endosperm, irregular divisions and nuclei of dissimilar sizes occur in the chalazal chamber. The favourable cytological properties

of *T. grandiflorum* permit a closer acquaintance with the nature of these two phenomena. Counts of metaphases show that polyploid chromosome numbers are relatively frequent. Metaphases with $6n = 30$, $9n = 45$, and $12n = 60$ could be observed. The chromosomes of these polyploid metaphases appear to be unchanged, as far as the insufficient starvation effect permits such an analysis. Presumably it is a matter of products of endomitotic divisions.

A second abnormality in the behaviour of the mitoses is shown particularly in the anaphases and telophases. Very frequently bridges and dumb-bell shaped nuclei are observed which prove that spontaneous chromosome breakage and reunions are not rare. It is possible that such persistent bridges occasionally give rise to the formation of polyploid nuclei.

Spontaneous chromosome breakage and polyploid mitoses also appear in the micropylar endosperm, but there they are rarer. In respect of these two phenomena there is consequently only a difference in degree.

The divergence between the chalazal and micropylar endosperm is accentuated with the increasing age of the seeds insofar that the intensity of division of the micropylar nuclei is significantly greater than that of chalazal. And the capacity for division lasts longer there. The micropylar endosperm therefore grows more quickly and also attains a greater bulk. With the beginning of the cell-wall formation, which can be seen only imperfectly in our leuco-basic fuchsin preparations, the mitoses cease to be simultaneous. They are irregularly scattered through the endosperm and their distribution is the same as in the meristems of the root-tip. The end of the synchronisation of the mitoses begins sooner in the chalazal chamber, which presumably means that the cell-wall formation starts sooner.

5. CYTOLOGY OF THE ENDOSPERM

(a) Metamorphosis of heterochromatin

In the resting nucleus, both with and without cold-treatment, there appear strongly stained chromocentres whose number varies from cell to cell. Darlington and La Cour (1941) assume that it is a question of the interkinetic condition of the H-segments. Their assumption was confirmed by the researches of Kurabayashi (1952*b*). According to him the larger chromocentres represent fusion products in which the H-segments of several chromosomes take part. So it is understandable that the number of chromocentres is variable and does not correspond to the number of heterochromatic blocks. The results of my own investigations are in close agreement with this discovery. Counts of chromocentres show that their proportion in the nuclei of the triploid endosperm and the diploid root cells is approximately 3 : 2.

In the endosperm preparations the H-segments of the prophase chromosomes are drawn out into very long thin threads (fig. 3).

These stages could not be observed in the cold-treated root-tip cells or in endosperm prophases at normal temperature. Two conclusions may be drawn :

(1) The long thin threads represent a cold effect which means that the spiralisation of the early prophase chromosome does not take place in the H-segments.

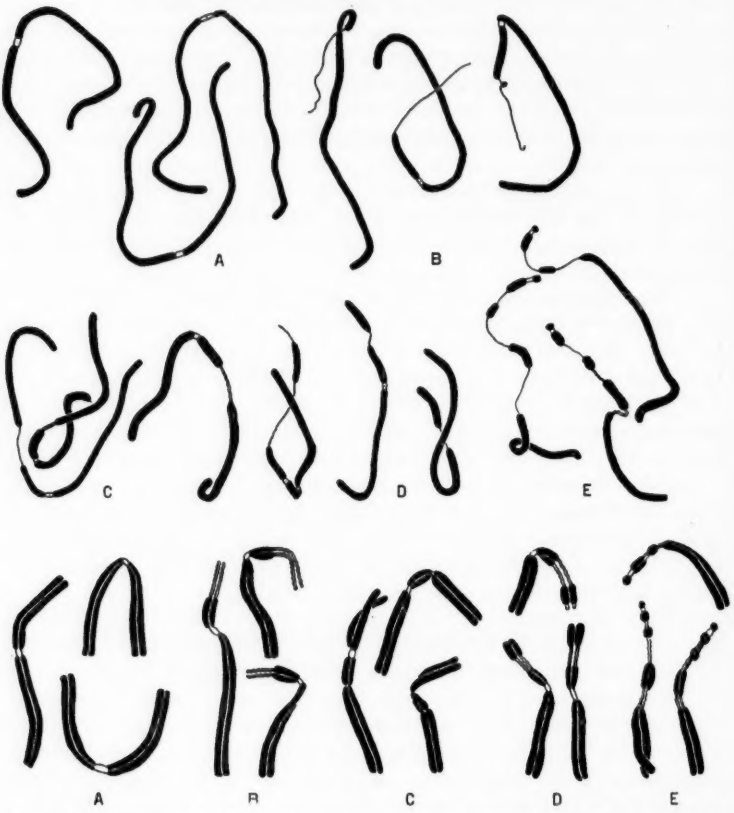

FIG. 3.—Chromosomes of chilled endosperms. Upper two rows : the 15 chromosomes of a late prophase. Third row : the 15 chromosomes of a metaphase plate. × 1250.

(2) The difference between the cold-treated prophases of the root meristem and the endosperm indicates that the behaviour of the heterochromatin is determined not only by the temperature but also by the immediate environment of the chromosomes.

The uncoiled state of the H-segments lasts throughout the pro-metaphase. Not until shortly before the metaphase do the H-segments

become shorter and thicker, but they never attain the same degree of condensation as in the root mitoses. And so the coiling apparently begins later and is not complete. Although the H-segments are now thicker than in the prophase, their stainability does not increase and so the difference in staining intensity between euchromatin and heterochromatin does not depend on differences in spiralisation. It is rather to be ascribed to a reduction in DNA content, as Darlington and La Cour (1940) have suggested.

It can clearly be seen from fig. 3 that the long thin segments of the prophase chromosomes correspond to the unstained regions of the metaphase chromosomes. There all chromosomes of a prophase and a metaphase which lie in the same endosperm have been drawn. The endosperm has the chromosome complement $A_0A_0A_0B_1B_1B_1C_1C_1C_1$ $D_1D_1D_1E_2E_3E_3$.

The reduced spiralisation in the H-segments of the prophase and metaphase chromosomes presumably is the reason that small H-segments are often more clearly visible in the endosperm chromosomes than in the metaphase chromosomes of the root-tips. The difference is greatest in the young endosperm.

(*b*) *Distribution of chromosomes*

The great variability of the H-segments of different individuals of the same sample stands in sharp contrast to their constancy within the root-tips of the same plant. Apart from the unstable H-segments only slight differences have been found between corresponding chromosomes of different mitoses, in relation to the extent of the heterochromatic blocks. Differences in their number and position do not appear at all. The question therefore arises whether the H-segments are changed in the course of seed formation and fertilisation and how the variability of the *Trillium* chromosomes arises. To decide this question individuals of *T. grandiflorum* were crossed and the F_1-endosperms tested. It is to be noted in all these tests that :

(1) the seed plants contribute two chromosome-sets to the chromosome complement of the endosperm nuclei, and that
(2) these two sets, because of the *Allium* type of the embryo-sac development, derive from a single dyad-cell.

Forty crosses were made and the chromosome complements of 717 endosperms were ascertained. The number of all chromosomes investigated was 33,354. They were distributed among 2207 mitoses. For the question of the constancy of heterochromatin the crosses between plants with *homomorphic* chromosomes are of especial importance. If we for the time being disregard the phenomena of spontaneous chromosome breakage (which is treated in a special chapter) it appears from my investigations that the H-segments are carried over exactly like the euchromatic segments, with astonishing regularity. In number and position the large H-segments always correspond exactly to those

in the root meristems. They undergo no change in this respect in the course of meiosis and gamete formation.

On the other hand occasional extra H-segments appeared in the course of endosperm development. Among more than 6000 E_2-chromosomes of the cross $E_2E_2 \times E_2E_2$ six such aberrations were found. The most frequent are shown in fig. 7 E, *b*. They reveal, besides the two normal H-segments, a short extra heterochromatic part that is inserted at the proximal end of the distal euchromatic region. The origin of the extra heterochromatic region can in these cases not be ascertained. However it is very probable that it is a question of an inversion (*cf.* Haga and Kurabayashi, 1954).

The two abnormal chromosomes in fig. 7 E, *c* and 8 *c* represent E_3-chromosomes. Their extra H-segment is exactly in the position occupied by the third H-segment in normal E_3-chromosomes. The discovery is worth noting that many E_2-chromosomes of the same

TABLE 3

Non-disjunction in endosperms of Trillium grandiflorum ($3x = 15$)

Chromosome type involved	Chromosome number			
	14	15	16	17
	...	710	...	...
A . . .	...	...	1	...
B . . .	...	...	1	...
D . . .	...	...	1	...
E . . .	1	...	2	1
Total . .	1	710	5	1

cross show a small incision in the same place. As the extra heterochromatic blocks are fairly broad, they can hardly be regarded as unstable H-segments, which have become visible because of uncoiling. We are rather inclined to interpret them as duplications of small heterochromatic regions already existing. Similar chromosome aberrations can also be observed in the C- and D-chromosomes. But they always appear singly only.

Non-disjunction. A striking, if very rare abnormality in behaviour is that, occasionally in an endosperm, 14, 16 or 17 chromosomes appear instead of the triploid number 15 (table 3). In all mitoses of such endosperms the same chromosome was always present in either the more numerous or the less numerous group. Four times the E-chromosome is concerned, the A-, B- and D-chromosomes once each (fig. 4*a*, *c*). The deviation can be explained by assuming that in the process of gamete formation non-disjunction has taken place. For the endosperms with 5 E-chromosomes (fig. 4*b*) it can be deduced with certainty that this procedure has taken place during the first

meiotic division of the EMC. If 2 E-chromosomes reach the surviving dyad cell, then all nuclei of the female gametophyte (including the two polar nuclei) each contain 2 E-chromosomes. In the endosperm

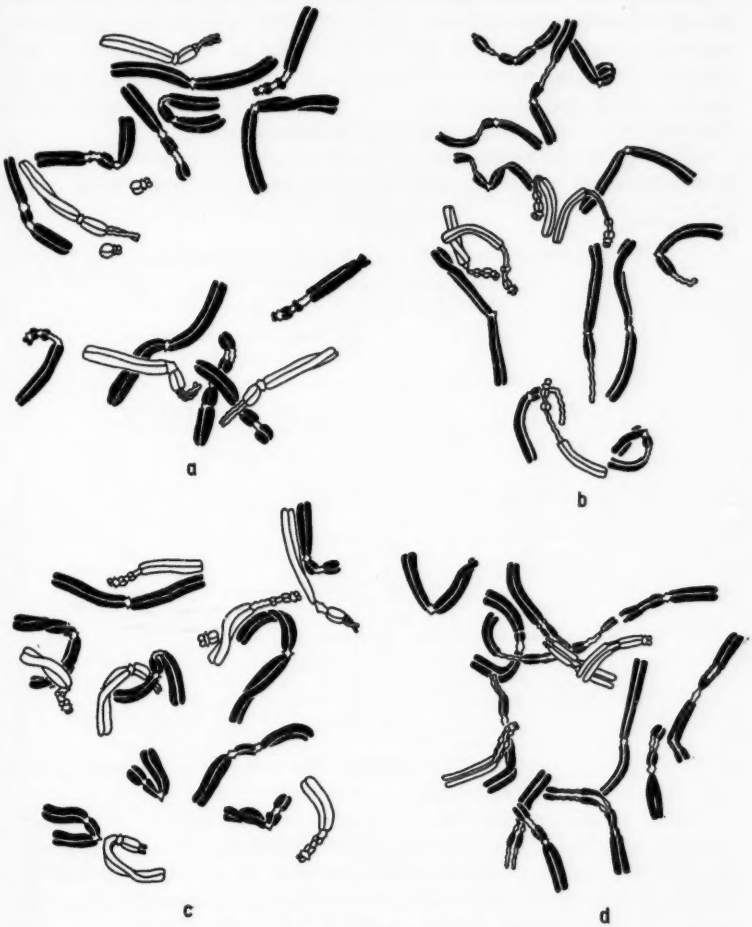

FIG. 4.—Metaphases of chilled endosperms :—(*a*, *c*) Non-disjunction on the male side, involving the B-chromosome (*a*) and the E-chromosome (*c*). (*b*) Non-disjunction on the female side (5 instead of 3 E-chromosomes). (*d*) Metaphase of the cross $E_1{}^3E_2 \times E_2E_2$ with two $E_1{}^3$- and one E_2-chromosomes. In (*a*) two, in (*c*) one fragment chromosome. ×1000.

then there must be 5 of them present, 4 from the female side and one from the male side. All endosperms with 14 or 16 chromosomes respectively, indicate non-disjunction on the male side and all endosperms with 17 chromosomes, non-disjunction on the female side.

The ratio discovered (6 : 1) indicates that in the course of pollen development more non-disjunction takes place, or else that supernumerary chromosomes are tolerated more readily in the pollen grains.

During the endosperm development non-disjunction also takes place. Occasionally mitoses with 14 or 16 chromosomes appear together with those which contain 15. But as there is always the danger of displacement of chromosomes in squash-preparations of tissues whose cells form only very thin membranes, somatic non-disjunction has not been quantitatively investigated.

Distributions of heteromorphic chromosomes and crossing-over. Crosses between plants with heteromorphic chromosome pairs must give

TABLE 4

Chromosome distribution of heteromorphic chromosome pairs.

(a) *Male plant heterozygote*

Cross	Chromosome complement of F_1-endosperms	
(1) $C_1C_1 \times C_1C_2$ . . .	$C_1C_1C_1$	$C_1C_1C_2$
G 2×G 3 . . .	4	9
G 19×GH 6 . . .	2	4
G 23×GH 25 . . .	5	3
G 46×GH 25 . . .	4	5
G 56×G 55 . . .	1	3
G 72×GH 25 . . .	1	8
GH 3×GH 6 . . .	1	0
GH 11×GH 2 . . .	1	3
Total	19	35
(2) $E_1E_1 \times E_2E_3$ . . .	$E_1E_1E_2$	$E_1E_1E_3$
GH 11×GH 2 . . .	1	1

different results, according to whether the male or the female plant contains the heteromorphic pair.

Since 4 microspores develop from meiosis, each male gamete contains only one of the 4 chromatids of a bivalent. The pollen grains of a C_1C_2 plant for example should therefore be half C_1 and half C_2. In a cross of the type $C_1C_1 \times C_1C_2$ one would have to expect endosperms with the chromosome complement $C_1C_1C_1$ and $C_1C_1C_2$ in equal numbers. The only cross of this type which permitted enough analyses, $C_1C_1 \times C_1C_2$, proved extremely abnormal : 19 endosperms $C_1C_1C_1$ oppose 35 endosperms $C_1C_1C_2$. The deviation from the ratio 1 : 1 is significant ($P < 0{\cdot}01$). It cannot be ascertained whether this deviation results from competition between C_1 and C_2 gametes or whether the viability of the endosperms depends to a certain extent on the chromosome constitution. Whichever influence is effective, one thing seems to be clear : the difference between the two

C-chromosomes, which presumably concerns their heterochromatin content has a clearly demonstrable physiological effect. Pollen grains or endosperms with a C_2-chromosome are favoured.

The results of the crosses between seed-plants with heteromorphic, and pollen plants with homomorphic, chromosomes permit a quantitative analysis. In part these results have been published elsewhere

TABLE 5

Chromosome distribution of heteromorphic chromosome pairs.

(b) *Female plant heterozygote*

Cross	Chromosome complement of F_1-endosperms			Frequency of post-reductions per cent.
(1) $C_1C_2 \times C_1C_1$.	$C_1C_1C_1$	$C_1C_1C_2$	$C_1C_2C_2$	
G 3×G 4 .	...	20	3	...
G 3×G 42 .	3	15	4	...
G 55×G 42 .	6	27	7	...
GH 1×G 19 .	...	1	1	...
GH 2×G 19 .	...	4	...	...
GH 6×G 45 .	1	7	2	...
GH 13×G 49 .	...	4	...	...
Total . .	10	78	17	74
(2) $E_2E_3 \times E_2E_3$.	$E_2E_2E_2$	$E_2E_2E_3$	$E_2E_3E_3$	...
G 40×G 4 .	3	8	6	...
G 40×G 23 .	2	6	...	...
G 72×G 45 .	4	11	3	...
G 72×GH 25 .	1	5	3	...
GH 2×G 19 .	1	3	...	...
GH 13×G 49 .	...	...	3	...
GH 20×G 45 .	...	1	1	...
GH 27×G 45 .	2	...	...	...
Total . .	13	34	16	54
(3) $B_1B_{1/4} \times B_1B_1$.	$B_1B_1B_1$	$B_1B_1B_{1/4}$	$B_1B_{1/4}B_{1/4}$	...
G 23×G 36 .	4	57	7	84

(Rutishauser, 1955*a*, *b*). But as several further cross experiments of this type have been analysed in the meantime, all results have been collated again in table 5. In all 3 cross combinations endosperms of the type A A A, A A a and A a a appear, where A stands for the B_1, C_1 or E_2 chromosome, a for $B_{1/4}$, C_2 or E_3. The appearance of 3 endosperm types can only be understood if we assume that :

(1) in the course of meiosis crossing-over takes place between the centromere and the point of the two homologous chromosomes where the difference begins, and

(2) that the division products of the first meiotic division are not separated, or, in our case, reunite after separation.

Fig. 5 reproduces these processes which take place during meiosis and embryo-sac development. The cross $E_2E_3 \times E_2E_2$ was chosen as an example (*cf.* also Rutishauser, 1955*a*, *b*).

Respecting the position of the chiasmata two possibilities are conceivable :

(1) An exchange takes place between the centromere and the difference. This is the case, according to the chiasma type theory, when a chiasma is in the section. Then two chromatids E_2E_3 reach the two dyad nuclei. They are separated (post-reduction) in the second meiotic division (which corresponds to the first step of division in embryo-sac development) but reunite again at the fusion of the polar nuclei. An E_2-chromosome is supplied by the sperm-nucleus, and so all endosperms have the chromosome complement $E_2E_2E_3$.

(2) In the chromosome part which is bounded by the centromere and the place where the difference begins, there is no exchange. The chiasmata lie outside this section. Then either two E_2 or two E_3 chromatids (pre-reductions) reach the surviving dyad cells. All nuclei of the embryo-sac consequently contain only E_2- or only E_3-chromosomes. After fertilisation with an E_2-chromosome endosperms with the constitution $E_2E_2E_2$ or $E_2E_3E_3$ must be expected in equal numbers.

As can be seen from table 5 that is the case, at least as far as the cross $E_2E_3 \times E_2E_2$ is concerned. The pre-reduction types of the cross $C_1C_2 \times C_1C_1$ (the $C_1C_1C_1$ and the $C_1C_2C_2$ endosperms) do not deviate noticeably from the ratio 1 : 1 ($P < 0{\cdot}01$). But it is striking that the number of the endosperms which carry double quantities of C_2-chromosomes and which therefore derive from a central cell C_2C_2, is higher than that of the $C_1C_1C_1$-endosperm.

It is to be seen that it is possible to deduce from the chromosome constitution of the endosperms whether the heteromorphic chromosome pairs have been pre- or post-reduced. All $E_2E_2E_2$- and $E_2E_3E_3$-endosperms arise through pre-reduction, but the $E_2E_2E_3$-endosperms through post-reduction.

The results of the crosses between plants with heteromorphic and homomorphic chromosome pairs can be checked by cross experiments on plants which are both heterozygote in the same chromosome pair. On the basis of the cross results given above, four different F_1-endosperms must appear, in the cross experiment $C_1C_2 \times C_1C_2$ for example $C_1C_1C_1$, $C_1C_1C_2$, $C_1C_2C_2$ and $C_2C_2C_2$ (table 6).

Both homozygous endosperms $C_1C_1C_1$ and $C_2C_2C_2$ as well as both heterozygote endosperms $C_1C_1C_2$ and $C_1C_2C_2$ should appear in the ratio 1 : 1. The number of post-reductions can be calculated from

this cross combination also. As is shown in table 6, only the heterozygous endosperms of the middle column are post-reductions. In the total number of the $C_1C_1C_2$-endosperms as well as in the total

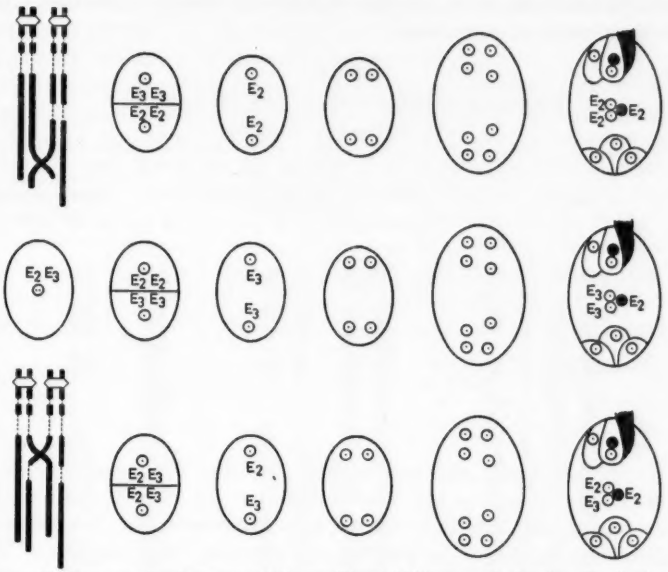

FIG. 5.—Embryo-sac development of *T. grandiflorum* (Allium-type) showing distribution of heteromorphic chromosome pairs (see text).

number of the $C_1C_2C_2$-endosperms there are included also pre-reductions. Their number is the same as the number of homozygous endosperms. It follows that the number of post-reductions can be

TABLE 6

Chromosome complements of F_1-endosperms of the cross $C_1C_2 \times C_1C_2$

♂ \ ♀	C_1C_1	C_1C_2	C_2C_2
C_1	$C_1C_1C_1$	**$C_1C_1C_2$**	**$C_1C_2C_2$**
C_2	$C_1C_1C_2$	**$C_1C_2C_2$**	$C_2C_2C_2$

(Post reductions are printed in heavy type)

calculated from the difference between the sum of all heterozygous endosperms and the sum of all the homozygous ones. The number of endosperms obtained by crosses of this type is still very small. As table 7 shows, all four expected chromosome complements nevertheless appear.

The frequency of post-reductions arrived at in table 7 amounts to 57 per cent. It does not agree with the value found for the cross $C_1C_2 \times C_1C_1$. But the difference is not significant ($P > 0{\cdot}01$). Further investigations will show whether agreement between the two cross-combinations is present or not.

TABLE 7

Chromosome distribution of heteromorphic chromosome pairs.

(c) *Both plants heterozygote*

Cross	Chromosome complement of F_1-endosperms				Frequency of post-reductions per cent.
(1) $C_1C_2 \times C_1C_2$.	$C_1C_1C_1$	$C_1C_1C_2$	$C_1C_2C_2$	$C_2C_2C_2$	
GH 6 × GH 25 .	2	5	3	...	...
GH 22 × GH 2 .	...	2	1	1	...
Total . .	2	7	4	1	57

From the results obtained with B-, C- and E-chromosomes it is evident that chiasmata are formed between the centromere and the place where the difference of the two heteromorphic chromosomes begins. Naturally, it is possible to measure these regions. The measurements are given in table 8 together with the corresponding values for the post-reductions.

TABLE 8

Comparison of the frequency of post-reductions and the measured centromere distance

Chromosome	Length of measured centromere distance		Frequency of post-reductions per cent.
	eu+het	eu	
B	4·3	3·1	84
C	8·1	5·6	74
E	10·9	5·4	56

As can be seen, there is no parallel between the frequency of post-reductions and the length of the measured centromere-distance, either the total length, or that of the euchromatic regions alone. Indeed the highest value coincides with the shortest distance. From these comparisons it is evident that the number of post-reductions does not tell us anything about the absolute length of the measured

centromere-distance, when various chromosomes are compared with one another. An agreement is only to be expected, as Darlington and Dark (1932) and Mather (1935) have already indicated, when the chiasmata happen to be distributed at random over the chromosomes. Reduction of the number of chiasmata or localisation of the chiasmata would essentially alter the picture.

The question of the position of the chiasmata could be solved if heteromorphic chromosome pairs were used which showed simultaneous differences in several places. Plants with such chromosome pairs occurred very rarely in my samples. One of them, GH 3, showed heteromorphic chromosomes of the type E_0E_3 (plate, fig. 1). It was crossed onto a plant with the chromosome constitution E_2E_2. Of

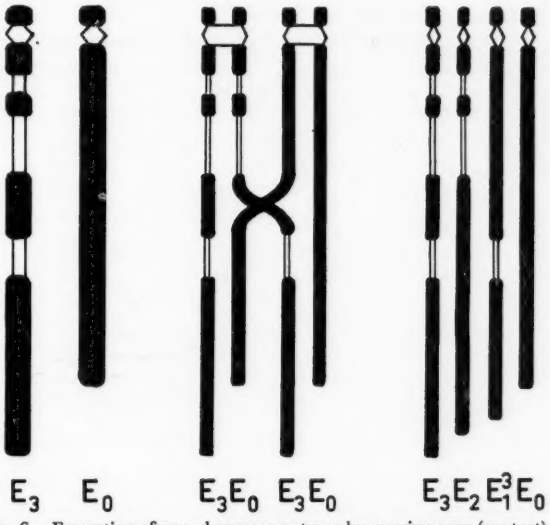

FIG. 6.—Formation of new chromosome types by crossing-over (see text).

the 11 endosperms which could be analysed in relation to the E-chromosome, 10 had the constitution $E_2E_2E_3$, and the eleventh endosperm showed a single strongly polyploid metaphase, in which a new E_1-chromosome appeared which could be found in neither of the two parents.

The same plant GH_3 was also crossed onto *Paris quadrifolia*. In one of the endosperms, whose mitoses contained 20 *Paris* and 5 *Trillium* chromosomes, the same chromosome could be observed again. It displays a single heterochromatic region, which lies in the place where in the E_3-chromosomes the most distal H-segment can be found (fig. 4*d*). There can be no doubt that it owes its origin to a chiasma which lay in the third euchromatic region (*cf.* scheme, fig. 6).

Unfortunately the number of tests is still too small to estimate

the frequency of the chiasmata in the various euchromatic regions of the E-chromosomes. However they show that the chromosomes with a greater number of H-segments are more easily transmitted than the others. Of a total of 11 endosperms which developed from a cross of GH3 and other *Trillium* specimens, 10 displayed the E_3-chromosome and only one the new $E_1{}^3$-chromosome.

It has been shown earlier (p. 369) that in the test plants of the GH sample two different E_1-chromosomes appear (fig. 1). One possesses a very short and often unstable heterochromatic region near the centromere; the H-segment of the other one, however, takes the place occupied by the third H-segment of the E_3-chromosome. This second type ($E_1{}^3$) corresponds with the E_1-chromosomes which developed from crossing-over. It is therefore very likely that it has the same origin. This interpretation is supported by the fact that in the same sample and only there E_0E_3-complements are also found. The abnormal $E_1{}^3$-chromosome was found in two plants, one together

Plate

FIGS. 1-4.—Metaphases of chilled root-tips.

FIG. 1.—Chromosome complement $A_0A_0B_1B_1C_1C_1D_1D_1E_0E_3$, E_3 at 3 o'clock, E_0 on the left.

FIG. 2.—Chromosome complement $A_0A_1B_1B_1C_1C_2D_1D_2E_1{}^3E_2$, C_1C_2 at 9 o'clock, $E_1{}^3$ at 10 o'clock, E_2 at 11 o'clock, A_1 centre.

FIG. 3.—Chromosome complement $A_0A_0B_1B_1/_4C_1C_1D_1D_2E_2E_2$, $B_1/_4$ at 7 o'clock.

FIG. 4.—Chromosome complement $A_0A_1B_1B_1C_1C_1D_1D_1E_0E_3$, A_1 at 3 o'clock, E_0 at 6 o'clock, E_3 at 12 o'clock.

FIGS. 5-8.—Metaphases of chilled endosperms.

FIG. 5.—Centric D-fragment (at 9 o'clock), two fragment chromosomes.

FIG. 6.—Isochromosome involving the short arm of a B-chromosome (at 5 o'clock).

FIG. 7.—E-chromosome with chromatid break (at 11 o'clock).

FIG. 8.—Normal metaphase of the cross $C_1C_2 \times C_1C_1$. Chromosome complement $A_0A_0A_0B_1B_1B_1C_1C_1C_2D_1D_1D_1E_2E_2E_2$.

with an E_2-, the other with an E_3-chromosome (plate, fig. 2). One of the two plants (with the complement $E_1{}^3E_2$) was cross-pollinated by an E_2E_2-plant. Two endosperms developed with the chromosome combination $E_1{}^3E_1{}^3E_2$ (pre-reductions). In both cases the $E_1{}^3$-chromosome was transmitted unchanged (fig. 4*d*).

(c) *Spontaneous chromosome breakage*

The subdivision of the cold-treated *Trillium* chromosomes into euchromatic and H-segments permits us to recognise morphological changes, *e.g.* losses and gains of euchromatic and heterochromatic substance, relatively easily. At the beginning of our investigations it was already clear that the chromosomes do not always retain their individuality in the course of endosperm development (Rutishauser, 1955*a*). Centric and acentric fragments, dicentric chromosomes, translocations and rings appear here and there in the mitoses, without an external cause being apparent. To a certain extent they resemble

E0
E3
B1
E3
E13
A1
C1
B1/2
D1
C2
D2
A0
B1
B1/4
E3
A1
E0
fr
D
fr
B
E2
C1
C1
C2

C2
C1
B
E2
D
B1/t
E0
E3
A1
B1
B1/t
E0
E3
C2
D2
A0
D1
C1
E3
E3
A1
B1

the chromosome fragments which have suffered chromosome breakage by the influence of mutagenic agents.

Types of aberrant chromosomes. In fig. 7 the most frequent types

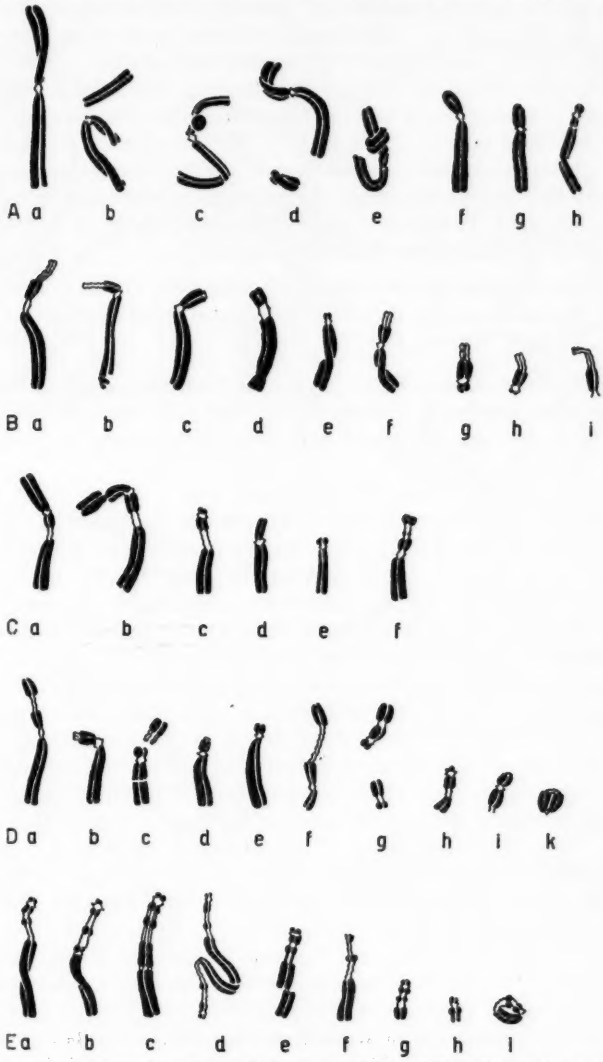

FIG. 7.—Types of centric and acentric fragments of *T. grandiflorum* (see text). ×1000.

of aberrant chromosomes are reproduced. For comparison a normal chromosome is shown also (fig. 7 A, *a*-E, *a*). It is clear that morphological changes can more easily be seen in such chromosomes as are

divided into many segments because of the arrangement of the heterochromatin. The E-chromosome is the best to analyse, the A-chromosome the worst.

By far the greatest number of aberrant chromosomes are centric fragments (plate, fig. 5). The breaks are found in the heterochromatin as well as in the euchromatin : any region is breakable. Of particular interest is the discovery that all chromosomes with the exception of the E-chromosome break at or in the centromere and give rise to the development of *telocentrics* (fig. 7 B, *i*, fig. 8*c*). As in X-ray-mitoses acentric fragments also appear beside centric fragments, and the fact that they belong to certain chromosomes is often easy to establish (fig. 7, fig. 8*e*). Dicentric chromosomes also appear in the mitoses. They come about in three different ways, viz. :

(1) by translocation between non-homologous chromosomes,
(2) by translocations between homologous chromosomes,
(3) by sister reunion (SR).

Dicentric chromosomes of type (1) are the most frequent. All chromosomes can be concerned in them (fig. 8*f*, 10*b*). Dicentric chromosomes which arise through SR, could only twice be observed in E-chromosomes (fig. 7 E, *d*, 8*d*).

SR apparently occurs only very rarely (fig. 7 A, *f*). Until now I have found only four dicentric chromosomes of type (2), three of which belong to the B- and one to the E-chromosome. Of particular interest is the aberrant B-chromosome in plate, fig. 6. It has only one centromere with two identical arms, and so has the appearance of an *isochromosome*. As it appeared with two B-chromosomes it is probable that it arose from a telocentric chromosome.

Translocations do not of necessity lead to the formation of dicentric chromosomes. Not infrequently centric and acentric fragments of different parentage unite too. But it is only rarely possible to conclude from the form of the changed chromosomes what the derivation of the acentric fragment is which has participated in the translocation.

Monocentric rings were observed only 5 times. Two of them were formed from the A-chromosome (fig. 8*a*), one each from a B-, D- (fig. 7 D, *k*) and E-chromosome (fig. 7 E, *i*). Acentric rings were somewhat more frequent. All 19 observed rings of this type contained only euchromatin (fig. 7 A, *c*, *e*).

Centric fragments represent the vast majority of all chromosome aberrations. But rarely fragmentations of chromatids can be observed as well. Some of them are reproduced in fig. 7 B, *b*, E, *e* and plate, fig. 7. Corresponding to their small number, the frequency of reunions between broken chromatids is extremely small ; only two doubtful cases were found.

Initial and secondary breakage. In reference to X-ray treatment, and depending on the time lapse, chromosome breakage of quite a different kind can appear (La Cour, 1952). Metaphases which

follow directly upon X-ray treatment (X_1 divisions) contain centric and acentric fragments, and also the bridges which are formed in

FIG. 8.—Metaphases of chilled endosperms. Spontaneous chromosome breakage (black : normal chromosomes, white : broken chromosomes and fragment chromosomes = fr). (*a*) ring of A-chromosome, centric D-fragment, 2 fr ; (*b*) centric A-, B- and E-fragment, 2 fr ; (*c*) telocentric C-chromosome, aberrant E_3-chromosome, 3 fr ; (*d*) dicentric E-chromosome, 1 fr ; (*e*) centric and acentric A-fragments ; (*f*) centric E-fragment, D-chromosome with attached acentric fragment of E-chromosome, 1 fr. × 1000.

the anaphases are accompanied by acentric fragments. As the latter have no centromeres, they are mostly eliminated and form micro-

nuclei in the telophase or are dissolved. The centric fragments can undergo SR and build bridges in the anaphases. The bridges usually break in two. So more chromosome breakages occur, but this time because of the forces the chromosomes are exposed to in the anaphase, and no longer under the immediate influence of ionising rays. In this process no acentric fragments develop. In the following metaphases and anaphases (X_2 divisions) therefore, as a rule only centric fragments appear.

By far the greatest number of all aberrant chromosomes of *T. grandiflorum* are unaccompanied by acentric fragments. It follows that we are dealing with fragments which were broken in a previous division. Only in a few mitoses could centric and acentric chromosomes side by side be discovered which derive from the same chromosome. In most cases it is a question of fragments of chromosomes, rarely of chromatids. The discovery that most reunions represent dicentric chromosomes which arose through translocation of non-homologous chromosomes also points to the greater frequency of chromosome breakage. So it is probable that the spontaneous chromosome breakage takes place before the division of the chromosomes during the interkinesis. Signs of subchromatid breakage (La Cour and Rutishauser, 1954) have not yet been found in these investigations.

The distribution of aberrant chromosomes. Most centric fragments occurring in endosperms of *T. grandiflorum* only appear in one metaphase plate. As there were frequently only a few nuclei of the same endosperm in division, which could be analysed, it cannot be stated when most aberrant chromosomes are formed and how often they are reproduced. In some cases, however, a more detailed analysis was possible.

As McClintock (1942*a*, *b*), Darlington and Wylie (1952), Hair (1952) and others have shown, the reproduction of centric fragments is often connected with a change of size if SR and bridge-formation preceded division, the secondary breakages do not always take place at the same point in the intercentric arm, and so unequal chromatids are distributed. According to McClintock (1939) the breakage-fusion-bridge cycle or SR-cycle only takes place in female gametophytes and in the endosperm. In most embryos, however, the broken ends " heal " and the centric fragments are passed on unchanged.

Another distribution procedure, called the R''-cycle by Darlington and Wylie (1952) takes place in maize (McClintock, 1942*a*, *b*) as well as in *Narcissus*. It concerns the division of dicentric chromosomes. If the intercentric arms in criss-cross position break, the broken ends of both chromatids can unite and form a new dicentric chromosome again. This process is the rule with *Narcissus* and the dicentric chromosome of this plant is therefore permanent.

The endosperms of *T. grandiflorum* seem to differ essentially from *Zea mays* in so far as the distribution of the centric fragments is concerned. SR is seldom seen in metaphases and anaphases ; the

centric chromosomes therefore divide without bridge building (fig. 10 *c*). As Brock (1955) supposed for *Hyacinthus*, McClintock's breakage-fusion-bridge cycle is not compulsory for all endosperms. Probably there is in the endosperm also a "healing" against SR. It may well be taken into account therefore that the centric fragments, at least in part, are transmitted unaltered. In good agreement with this supposition is the discovery that the same aberrant chromosome often appears in several metaphase plates of the same endosperm. Many of them, especially these which are derived from B- and E-chromosomes, could be found in 6 to 9 mitoses, which proves that they have survived at least 3-4 division cycles unaltered.

Dicentric chromosomes appear only very rarely in more than one metaphase plate. They form bridges, as is seen in the few anaphases with such chromosomes. They are divided parallel only very rarely. However, a dicentric D-chromosome which is presumably transmitted by a male gamete forms an exception. As can be seen from fig. 9

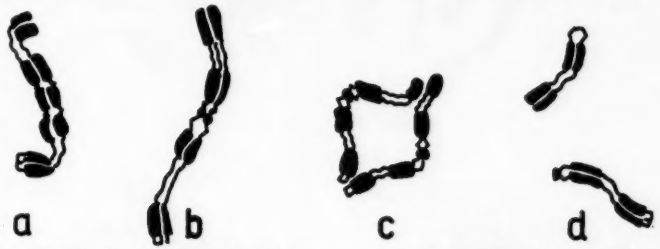

FIG. 9.—Distribution of a dicentric D-chromosome :—(*a*, *b*) two different types of dicentric D-chromosomes ; (*c*) anaphase, parallel separation of the dicentric ; (*d*) the two centric fragments of a broken dicentric. ×2000.

the two centromeres lie close to each other, as in the dicentric chromosomes of *Scilla peruviana* (Battaglia, 1949). They were found in 6 metaphase plates and so have been transmitted through at least 3 division cycles unaltered. The distribution of the dicentric chromatids is mainly parallel (fig. 9). Occasionally bridges may be formed which break in the intercentric arm, often near one centromere (fig. 9*d*).

As just mentioned, occasionally broken chromosomes also are carried over into the endosperm by the pollen. The breakages which produced them are here denoted gametic breakages to distinguish them from the somatic ones. The chromosome fragments which appear in all analysable mitoses of an endosperm are considered as gametic in origin. This occurrence is very rare. Among 717 endosperms only 5 contained gametic breakages, *viz.* 1 B and 4 D fragments. So only 0·7 per cent. at the most of all gametes have transmitted aberrant chromosomes. This figure is somewhat lower than should be expected from Sparrow's (1950) statements on the number of acentric fragments for microspores of *T. erectum*.

Frequency of chromosome breakage. Counts of over 2000 mitoses distributed throughout 717 endosperms have disclosed that 355 of 33,354 chromosomes showed breakage. A number of them, about 70-80, are demonstrably copies of the same broken chromosome. All

FIG. 10.—Metaphases and anaphase of chilled endosperms (black : normal chromosomes, white : broken chromosomes and fragment chromosomes) :—(*a*) hexaploid metaphase (note : all chromosomes are normal, 2 fr) ; (*b*) centric C-fragment, translocation between B- and C-chromosome, 1 fr ; (*c*) anaphase, normal distribution of centric B- and E-fragments (no SR). ×1000.

the others appear only once in the same endosperm. Therefore they can be taken as proof that at least one initial breakage has taken place. In order to ascertain the number of initial breakages in endosperms which show copies of centric fragments, the number of

TABLE 9

Frequency and distribution of spontaneous chromosome breakage in Trillium *endosperms*

TABLE 9

Frequency and distribution of spontaneous chromosome breakage in Trillium *endosperms*

Cross	No. of crosses	No. of end.	Chromosome types															Total		
			A			B			C			D			E					
			n.	br.	% br.	n.	br.	% br.	n.	br.	% br.	n.	br.	% br.	n.	br.	% br.	n.	br.	% br.
O×O	15	168	1433	10	*0·70*	1436	15	*1·05*	1445	2	*0·14*	1440	17	*1·18*	1444	8	*0·55*	7198	52	*0·72*
O×*f*	15	308	3025	21	*0·69*	2996	41	*1·37*	3018	12	*0·40*	3021	28	*0·93*	3024	70	*2·32*	15084	172	*1·14*
f×O	6	191	1842	19	*1·03*	1837	19	*1·03*	1830	6	*0·33*	1823	26	*1·43*	1851	36	*1·95*	9183	106	*1·15*
f×*f*	4	50	380	2	*0·53*	381	4	*1·05*	374	8	*2·14*	375	2	*0·53*	379	9	*2·38*	1889	25	*1·32*
Total	40	717	6680	52	*0·78*	6650	79	*1·19*	6667	28	*0·42*	6659	73	*1·10*	6698	123	*1·84*	33354	355	*1·06*

n. = no. of scored chromosomes

TABLE 10

Frequency and distribution of initial chromosome breakage in Trillium *endosperms*

Cross	No. of crosses	No. of end.	Chromosome types															Total		
			A			B			C			D			E					
			n.	br.	% br.	n.	br.	% br.	n.	br.	% br.	n.	br.	% br.	n.	br.	% br.	n.	br.	% br.
O×O	15	168	1433	9	*0·63*	1436	10	*0·70*	1445	2	*0·14*	1440	12	*0·83*	1444	5	*0·35*	7198	38	*0·53*
O×*f*	15	308	3025	19	*0·63*	2996	30	*1·00*	3018	10	*0·33*	3021	24	*0·79*	3024	40	*1·32*	15084	123	*0·82*
f×O	6	191	1842	14	*0·76*	1837	13	*0·71*	1830	6	*0·33*	1823	19	*1·04*	1851	25	*1·35*	9183	77	*0·84*
f×*f*	4	50	380	2	*0·53*	381	4	*1·05*	374	7	*1·87*	375	2	*0·53*	379	8	*2·11*	1889	23	*1·22*
Total	40	717	6680	44	*0·66*	6650	57	*0·86*	6667	25	*0·38*	6659	57	*0·86*	6698	78	*1·17*	33354	261	*0·78*

n. = no. of scored chromosomes

all identical aberrant chromosomes in each endosperm was reduced to one. In table 9 the frequency of all centric fragments including copies is given, and in table 10 the frequency of initial breakage. It is possible that the values for initial breakage are a little too high. Occasionally there were found in the same endosperm centric fragments of the same chromosome which differed from each other morphologically. As it cannot be decided for certain whether they arose from secondary changes, *e.g.* through asymmetric breakage of bridges or whether they should be regarded as signs of two different initial breakages, there is here some source of uncertainty.

With this reservation in mind we can make the following conclusion from the results obtained.

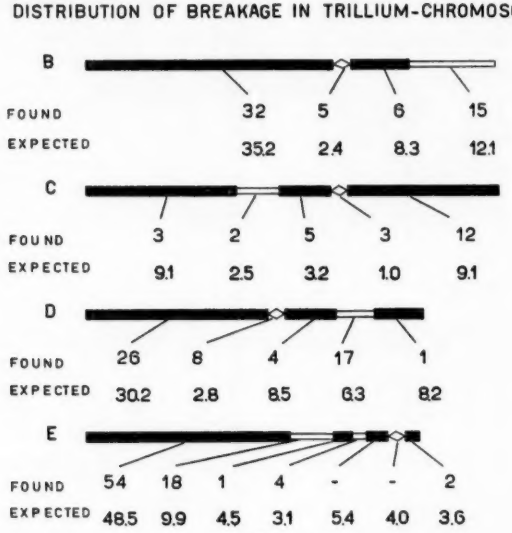
DISTRIBUTION OF BREAKAGE IN TRILLIUM-CHROMOSOMES
B
FOUND 32 5 6 15
EXPECTED 35.2 2.4 8.3 12.1
C
FOUND 3 2 5 3 12
EXPECTED 9.1 2.5 3.2 1.0 9.1
D
FOUND 26 8 4 17 1
EXPECTED 30.2 2.8 8.5 6.3 8.2
E
FOUND 54 18 1 4 - - 2
EXPECTED 48.5 9.9 4.5 3.1 5.4 4.0 3.6

FIG. 11.

(1) As a comparison of the relative breakage frequencies shows, as presented in tables 9 and 10, the corresponding values do not decline uniformly for all chromosomes. The difference amounts to 28 to 34 per cent. for the B- and E-chromosomes, and only about 10 to 22 per cent. for the others. That means that the centric fragments of B- and E-chromosomes are reproduced unchanged more frequently than those of the other chromosomes. The A- and C-fragments especially produced only a few copies. It must be noticed that the B- and E-chromosomes are strongly asymmetrical in respect of the position of the centromere. The fragments most often reproduced as a rule developed from a break in the long arm, which led to the formation of short chromosomes with a more median centromere position. It is conceivable that the reproductive ability of the

fragments is improved thereby. In this sense the position of the centromere seems to have an influence on the multiplication of centric fragments. It would also be possible for the centromere to prevent SR and so favour the development of copies.

(2) The breaks are unevenly distributed over the 5 chromosomes of the genome of *T. grandiflorum*. The E-chromosome is the most frequently broken, the C-chromosome least. Both synopses give the same picture, but with the difference that the results for initial breakages are somewhat more even. This discovery is reminiscent of the results obtained with radiomimetic substances (Ford, 1948; Revell, 1952; McLeish, 1952) where in contrast with the X-ray experiments, an uneven distribution of breaks in the various chromosomes was observed. In the *Trillium* experiments, however, the frequency of breakage has no relation either to the absolute length of the chromosomes or to their relative heterochromatin content.

(3) The distribution of breaks over the various regions of the B-, C- and E-chromosomes seems to be more or less random (fig. 11). Only the heterochromatic region of the D-chromosome is favoured for breakage. But it is not improbable that this uneven distribution comes to pass because of secondary changes.

In the experiments on the variability of chromosomes in the course of endosperm development three series of cross tests were included which were carried out on plants with fragment chromosomes. In tables 9 and 10 the four cross combinations O×O, O×*fr*, *fr*×O and *fr*×*fr* are given separately. It can be seen from the last column of the tables that the number of breaks varies according to the cross combination. It is smallest in the O×O crosses, greatest in the *fr*×*fr* crosses. This discovery gives the impression that there is a connection between frequency of breakage and number of fragment chromosomes. But in all three crosses in which *fr*-plants were included, there were endosperms with and without fragment chromosomes. In the O×*fr* cross there were some endosperms with one fragment chromosome and some without any in the ratio 1 : 1; in the remaining crosses endosperms appeared with 0, 1, 2 and 3 standard fragments. So it has proved necessary to calculate the number of centric fragments separately for all endosperm categories.

That has been done in table 11 for all broken chromosomes, in table 12 for the initial breaks. From them the following results may be derived: the breakage frequency in the endosperms without fragment chromosomes amounts to 0·80 per cent., rises to 1·30 per cent. in the endosperms with one fragment and remains at about this figure if more standard fragments are introduced.

Table 12, which gives the distribution of initial breakages, shows the same regularity. In this synopsis also the differences are significant in respect to the breakage frequency of the different endosperm categories ($P < 0{\cdot}001$). The 3 *fr*-endosperm, for which too few counts have been made is again an exception.

TABLE 11

Influence of fragment chromosomes on spontaneous chromosome breakage

Cross	No. of crosses	No. of end.	O-end.			*f*-end.			*ff*-end.			*fff*-end.			Total		
			n.	br.	% br.	n.	br.	% br.	n.	br.	% br.	n.	br.	% br.	n.	br.	% br.
O×O	15	168	7198	52	*0·72*	...	...	...	...	...	...	...	...	...	7198	52	*0·72*
O×*f*	15	308	7079	66	*0·93*	8005	106	*1·32*	...	...	...	...	...	...	15084	172	*1·14*
f×O	6	191	1185	5	*0·42*	133	0	*0*	7810	101	*1·29*	55	0	*0*	9183	106	*1·15*
f×*f*	4	50	164	2	*1·22*	44	0	*0*	956	13	*1·36*	725	10	*1·38*	1889	25	*1·32*
Total	40	717	15626	125	*0·80*	8182	106	*1·30*	8766	114	*1·30*	780	10	*1·28*	33354	355	*1·06*
						$\chi^2=13{\cdot}05$ P<0·001			$\chi^2=14{\cdot}38$ P<0·001			$\chi^2=2{\cdot}81$ P>0·05					

n. = no. of scored chromosomes

TABLE 12

Influence of fragment chromosomes on initial chromosome breakage

Cross	No. of crosses	No. of end.	O-end.			*f*-end.			*ff*-end.			*fff*-end.			Total		
			n.	br.	% br.	n.	br.	% br.	n.	br.	% br.	n.	br.	% br.	n.	br.	% br.
O×O	15	168	7198	38	*0·53*	...	...	...	...	...		...	...	...	7198	38	*0·53*
O×*f*	15	308	7079	47	*0·66*	8005	76	*0·95*	...	...	...	...	...	...	15084	123	*0·82*
f×O	6	191	1185	5	*0·42*	133	0	*0*	7810	72	*0·92*	55	0	*0*	9183	77	*0·84*
f×*f*	4	50	164	2	*1·22*	44	0	*0*	956	12	*1·26*	725	9	*1·24*	1889	23	*1·22*
Total	40	717	15626	92	*0·59*	8182	76	*0·93*	8766	84	*0·96*	780	9	*1·15*	33354	261	*0·78*
						$\chi^2=8{\cdot}59$ P<0·01			$\chi^2=10{\cdot}98$ P<0·001			$\chi^2=3{\cdot}39$ P>0·05					

n. = no. of scored chromosomes

The distribution of breaks over the 2207 analysed mitoses was only ascertained for 349 aberrant chromosomes. The remaining 6 centric fragments lay in mitoses which could not be fully investigated. Further, because of gametic and somatic non-disjunction, the number of investigated mitoses is somewhat lower than would be expected from the total number of chromosomes (33,354). As table 13 and fig. 12 show, the distribution of the aberrant chromosomes deviates only slightly from a Poisson distribution, from which it follows that

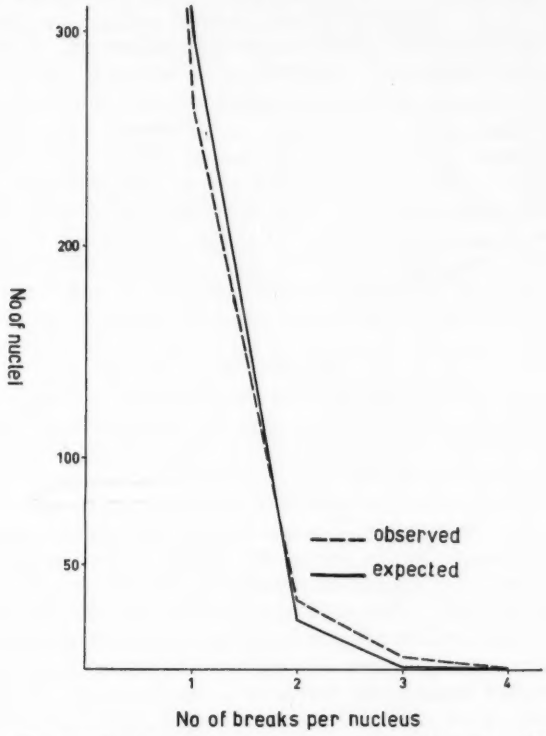

FIG. 12.—Frequency polygon of spontaneous breakage in *T. grandiflorum*.

the number of chromosome breaks is not raised essentially by previous breakages. Unlike Brock (1954) we can therefore find no indications of a snowball increase of breakage frequency.

From the figures presented in table 13 the frequency of aberrant chromosomes per metaphase amounts to 0·158. This figure is somewhat smaller than the total frequency of B″ and B′ breaks of *Trillium* roots given an X-ray dose of 5*r* as discovered by Darlington and La Cour (1945). In proportion to the frequency of spontaneous chromosome breakage in other plant organisms, however, it is

relatively high. So Giles (1940) found in microspores of *T. canaliculata* and *T. paludosa* frequencies of only 0·04 and 0·06 per cent. *T. erectum* on the other hand, according to Sparrow (1950) shows higher values also in PMC and microspores. But there the breaks which occur during the meiosis are not separated from those which occur during the first pollen grain mitoses. In root cells spontaneous chromosome breakage was only rarely found.

TABLE 13

Frequencies of breaks per nucleus

Classes	Observed	Expected
0	1905	1884·5
1	263	297·9
2	32	23·5
3/4	7	1·2
	2207	2207·1
Variance = 0·184		Mean = 0·158
$\chi^2 = 35{\cdot}44$		$P<0{\cdot}001$

6. DISCUSSION

(i) *Heterochromatin in the endosperm*

The cold-treatment experiments with the endosperm give further confirmation that the heterochromatin is influenced by the function of the cell as has been found in X-chromosomes of locusts. This is shown particularly clearly in prophase chromosomes of cold-treated plants. In the root-tips the H-segments are only slightly narrower than the euchromatic blocks during the prophase. But in the endosperms they appear as long, thin threads, in contrast with the broad euchromatic segments. Not until prometaphase are the H-segments thickened. So they are clearly late in coiling. The difference between root meristems and endosperms is presumably to be only quantitative. The cold-treated endosperms reveal only one property of the H-segments more clearly. They show that the H-segments are allocyclic in respect not only to their reaction to leuco-basic fuchsin, but also to spiralisation. In the resting nucleus the heterochromatin is strongly spiralised, the euchromatin weakly. The uncoiling of the H-segments, however, appears to be faster and more complete after cold-treatment. Further, the heterochromatin lags in respect to formation of a new spiral (see also Boothroyd, 1953).

The cold-treatment also has an influence on the reaction of the H-segments to Feulgen staining. The uncoiled heterochromatic prophase threads appear more lightly stained than the euchromatin. It is debatable whether this difference is due to the weaker staining intensity or whether it only appears so because of the smaller diameter

of the threads. Later, with increased spiralisation and shortening of the H-segments this uncertainty disappears. The reaction to Feulgen is then clearly negative or at least weakened in relation to the euchromatic regions.

The results given refer to the behaviour of the heterochromatin in the micropylar part of the endosperm only. In the chalazal region it is somewhat different. The endosperm nuclei of this section are always more darkly stained, as are the chromosomes of all mitoses stages. The cold effect manifests itself only incompletely. The difference in staining between euchromatin and heterochromatin is smaller in the metaphase stage, and particularly, the prophase chromosomes show no uncoiled regions. Euchromatin and heterochromatin are not distinguishable during the prophase, and in the metaphase plates the differences are blurred. From these observations it follows that the H-segments of the same plant, even of the same tissue, react differently to low temperatures.

The difference in the staining ability of micropylar and chalazal endosperm nuclei is parallel to a difference in cold effect. The H-segments are weaker stained in mitoses which derive from weakly stained nuclei, and the same is true for euchromatin also. If we take the staining intensity with leuco-basic fuchsin as an index of the deoxyribosenucleic acid (DNA) content it appears that the cold effect is not produced when originally there is much DNA present. This interpretation would agree with Darlington's conception, according to which heterochromatic regions are only revealed when the supply of DNA drops below a threshold value.

The more recent interpretation of DNA as an active genetic substance makes an exchange of DNA with different genetically active and inactive regions of the chromosome improbable. The assumption that the production of DNA is lowered by cold-treatment, whereby the H-segments react more sensitively to the changed environment is then more likely. The peripheral position of the chromocentres would also support such an interpretation (Vanderlyn, 1949). Or we have to assume with Darlington (1955) that there are different states of DNA.

(ii) *Cytological evidence of crossing-over*

The consequences of crossing-over can only be cytologically examined when the partners of a homologous chromosome pair are different. Deficiencies can serve as a landmark, or the normally present morphological differences between X- and Y-chromosomes can be used (Koller and Darlington, 1934; Matthey, 1936, but see also Matthey, 1952). Further, for such examinations the condition must be fulfilled that the two chromatids which come from the first meiotic division remain together or if they were divided, unite again.

The second condition is fulfilled if the division products of heteromorphic bivalents can be distinguished from one another clearly in

the anaphase of the first meiotic division. Such examinations have been carried out by Carothers (1913), Wenrich (1916), Darlington (1936), White (1949) and others, with locusts, and by Hearne and Huskins (1935) with *Melanoplus femur-rubrum* and Huskins and Spier (1934) with *Triticum vulgare*.

Three modes of behaviour of heteromorphic bivalents can be found : the first meiotic division is either purely reductional or purely equational or it is a mixture of both division types. That would mean that between the centromere and the point where the difference begins either a chiasma is formed always or never, or that finally chiasmata sometimes appear and sometimes not.

The reason for the different meiotic behaviour of heteromorphic bivalents depends on their structure, according to Mather (1935) and Darlington (1937). If the difference lies close to the centromere, no chiasma is formed proximal to it, the division is always reductional (pre-reduction). If the difference lies on the opposite end, and if only one chiasma is formed, the bivalents split equationally (post-reduction). So it is the position of the difference, or rather its distance from the centromere which determines the mode of reduction.

With plants of the *Allium* type of embryo-sac development it is possible to examine the cytological consequences of crossing-over in the endosperm. The two chromatids of a dyad are separated in the course of embryo-sac development. They are distributed to the two nuclei of the two-nuclear embryo-sac, each of which forms a quartet of haploid nuclei. Later two nuclei of the two quartets fuse together and thereby the two chromatids are brought together again.

A similar procedure has already been used by Goldschmidt (1932) to discover whether post- or pre-reduction takes place. In *Bombyx mori* the second polar body is occasionally not ejected, but remains in the egg and like the egg nucleus is fertilised by a sperm nucleus. If the two nuclei are different, mosaics appear. Gynandromorphs, for example, are formed when the X- Y-chromosome pair split equationally. In this case too, it is assumed that the values obtained for the frequency of pre-reductions depend on the distance between the difference and the centromere.

The cytological analysis of the endosperm from crosses of plants with heteromorphic chromosome pairs makes possible further statements about the mechanism of crossing-over. In these examinations only chromosomes which differed in number and position of heterochromatic blocks took part. The euchromatic regions of the heteromorphic chromosome pairs are always equally long. As cross experiments of the type $C_1C_1 \times C_1C_2$ show, the difference in heterochromatin content has a physiological effect which must be taken into account in the interpretation of the results : endosperms of the constitution $C_1C_1C_1$ and $C_1C_1C_2$ appear approximately in the ratio 1 : 2 instead of 1 : 1, *i.e.* either the gametes or the endosperms with C_2-chromosomes are favoured. The same effect is also found when E_0- and E_3-chromo-

somes compete with one another. In the E_2E_3 experiments, however, the effect is lacking.

The frequencies of post-reductions presented in table 5 are correct for the combination $E_2E_3 \times E_2E_2$, but they are presumably too great for the combination $C_1C_2 \times C_1C_1$ and $C_1C_2 \times C_1C_2$, supposing that on the female side, too, gametes or endosperms with C_2-chromosomes are favoured. It cannot be stated for certain whether the results of the $B_1B_{1/4} \times B_1B_1$ also have been changed by a physiological effect of the heterochromatin. But even when the influence operates in reverse there (favouring the $B_{1/4}$ gametes or endosperms and thereby raising the values for post-reduction) the results of our experiments would still hold: the frequency of post-reduction is not proportionally dependent on the distance between difference and centromere. The highest value coincides with the shortest distance.

Darlington (1937) and Mather (1937) have already shown the disproportion between the size of chromosomes and number of chiasmata. The discrepancy is particularly great where the size difference between the chromosomes is marked, as for example in *Stenobothrus parallelus* (Darlington and Dark, 1932) and *Melanoplus femur-rubrum* (Hearne and Huskins, 1935). Darlington and Mather attribute the phenomenon to the fact that the points at which crossing-over can occur are localised. Localisation of chiasmata is cytologically proved for *Mecostetus*, *Fritillaria meleagris* and other organisms. According to Mather's (1937) view it is, however, general. In his opinion the first chiasma always occupies a definite region of the chromosomes. This region, which is designated " differential distance ", lies near a landmark of the chromosome, *e.g.* the centromere or the distal end of the chromosome. If the chromosome exceeds the length of the differential distance, further chiasmata can be formed at intervals determined by interference.

On the basis of this interpretation of chiasma distribution the results of my experiments can be understood. Assuming that the first chiasma of both chromosome arms always lies near the centromere (proximal localisation) and that because of this the frequency of chiasma formation is greater in this region than at other places in the chromosome, it is understandable why there is no correlation between measured centromere distance and chiasma frequency. If the distance between difference and centromere is short, as in the short arm of the B-chromosome, only one chiasma is formed on this length. The frequency of post-reductions is therefore high. If, with an increased interval, as for example in an E-chromosome, another chiasma appears, the number of post-reductions falls, because then part of the crossover is cancelled by the second chiasma. The greater distance from the centromere must therefore lead first to a decline in the frequency of post-reductions. It does not rise again until more chiasmata are formed.

Unfortunately there are at present no analyses of meiotic divisions

for *T. grandiflorum* which could be used in comparison with the results obtained with endosperms. According to Huskins and Smith (1935, 1938) the number of chiasmata per nucleus for *T. erectum* is relatively small. It varies between 14·4 and 17·15 according to the phase of division investigated, and may therefore amount to not more than three on the average for each chromosome pair. The chiasmata do not appear to be localised. Proximal localisation of chiasmata, however, has been ascertained for *T. kamtschaticum* by Matsuura (1937). I shall not go into his interpretation of the meiosis of this plant (neo-two-plane theory) until more results of the cross $E_0E_3 \times E_2E_2$ are at hand.

From the work of Darlington and La Cour (1941), Kurabayashi (1952) and Haga and Kurabayashi (1954) as well as from my own investigations it is clear that the structure of the *Trillium* chromosomes is extremely variable. Number, position and length of the heterochromatic regions—and occasionally the euchromatic regions too—change even between individuals of the same population. Haga and Kurabayashi have shown that this variability in the last resort goes back to chromosome breakage and consequent reunions. They succeeded in discovering inversions and translocations in *T. kamtschaticum* and the results of my own experiments point in the same direction. But they point out yet another source of the diversity of forms: new chromosome types can arise from crossing-over, provided that heteromorphic chromosome pairs are present. So a new E_1-chromosome (E_1^3) developed from the homologous chromosome pair E_0E_3, the H-segment of which was distal instead of being proximal. All remaining discovered chromosome types (the E_1^1- and E_2-chromosomes) can be derived from the same heteromorphic chromosome pair by changes in the number and position of the chiasmata.

(iii) *Spontaneous chromosome breakage*

Structural changes of chromosomes can appear in different places in the nuclear cycle. According to Darlington's chiasma-type theory chromatid breakages and reunions normally appear during prophase of the first meiotic division. As a rule they only lead to visible structural changes if the homologous chromosomes show inversions following previous breakages. From these chromosome aberrations of structural hybrids we must distinguish sharply the chromosomal changes appearing in the course of mitoses. These alone are termed spontaneous chromosome breakage here.

Isolated references to the appearance of spontaneously developed aberrant chromosomes and their behaviour in the course of development are frequent in literature. But there have been very few experiments which could throw light upon the nature and time of their origin. It seems to be clear from the results to hand that not all tissues are equally subject to the phenomenon of spontaneous chromosome breakage. By far the most breakages have been found in gametophytes,

especially in pollen grain mitoses (Darlington and Upcott, 1941 ; Giles, 1940-41 ; Sparrow and Sparrow, 1950) in embryo-sacs (Haga and Kurabayashi, 1954) and also in ovular tissues (Kurabayashi, 1952) and recently also in the endosperm (Rutishauser, 1953, 1954*a* : Brock, 1953, 1954). On the other hand they are relatively rare in mitoses of root-tips.

With *T. grandiflorum* the experiment was made to determine quantitatively the breakage frequency in the endosperm and in the root tissue. As this species is highly self-sterile, both tissues derive from cross-pollinated seeds. In 2129 chromosomes of root mitoses, including those with fragment chromosomes, not a single aberrant chromosome was found, and in 33,354 chromosomes of endosperms, on the other hand, there were 355. Thus the breakage frequency differs in these two tissues. Kurabayashi (1952) comes to the same conclusion: in *T. kamtschaticum* spontaneous chromosome breakages occur occasionally in the ovular tissue (in one plant in 23 per cent. of all cells) but very rarely or never in root-tips. As chromosome breaks are connected with losses or at least with transposition of genetic substances, it is to be assumed that the somatic mutability also is dependent on the tissue. That has already been shown in the case of the endosperm of maize. According to Jones (1936*a*) the number of seeds with mosaics in ccC-endosperms is more than 25 per cent. (C = basic factor for colour). The mosaic rate per cell is reckoned at 1 : 70,000. But that of spontaneous gene mutation c→C in gametes is with 1 : 14 millions considerably lower (Jones, 1936*b*).

Different suppositions have been made about the cause of the increased appearance of chromosome breakage in many plant tissues. Kurabayashi (1952) attributes the phenomenon to a disturbance of genetic balance. But where the chromosome complement is balanced he supposes a disturbance of the mechanism which is connected with the chromatin increase. Brock (1954) believes that the endosperm, where particularly many spontaneous chromosome breaks occur, reacts more sensitively in relation to other tissues. In my view it is conceivable and even probable that the higher "sensitivity" of the endosperm is only simulated. The higher breakage frequency could come about simply because in the endosperm mutagenic agents are formed or accumulated more easily than elsewhere. The endosperm has a nutritional function, so its metabolism must be different from that of a root-tip cell. The same is also true of the ovular tissue and the tapetum.

The factors which cause chromosome breakage are still unknown. The natural ionising rays are not sufficient for their causation. As a rule a disturbance of genetic balance or of cell metabolism is held responsible. As especially Giles (1940-41) was able to show with a very large number of samples, the breakage frequency in the pollen grains of hybrid species is higher than in the parents. In the F_2 hybrids between *Tradescantia canaliculata* and *T. humilis* it increases

threefold and is still higher in triploid hybrids between *T. canaliculata* and *T. paludosa*. Giles attributes this increase of spontaneous breakage frequency to a disturbance of the balance of the chromosome complement. He observes mistakes in the coiling cycle and believes that genes which disturb the normal timing of the coiling cycle are accumulated by the cross. Brock too (1955) tries to explain the high breakage frequency in endosperms of hyacinths by disturbances in the balance. The addition of a long chromosome with nucleolus organiser raises the breakage frequency more sharply than when 2 L^n chromosomes are introduced. The decisive investigation, however, has only been carried out on a single endosperm and therefore cannot be accepted as validated.

In another work (Rutishauser and La Cour, 1955) it has been shown that even hybridisation between species of different genera does not always lead to an increase in breakage frequency. The breakage frequency in the endosperms of the cross *Paris quadrifolius* × *T. grandiflorum* is twenty times greater than in the endosperms of the reciprocal crosses and in the latter not much higher than in pure *Trillium* endosperms. So it is not probable that the hybrid constitution or the disturbance of the chromosome balance in itself alters the breakage frequency. Rather would it appear that special conditions must be fulfilled for the induction of spontaneous chromosome breakage.

The experiments with fragment chromosomes point in the same direction. It has been stated above in some detail that the breakage frequency in endosperms with 1, 2, or 3 fragment chromosomes is distinctly higher than in endosperms with none (1·3 per cent. as opposed to 0·80 per cent.). This result could also mean of course that a disturbance of the genetic balance is caused by the fragment chromosomes. Fragment chromosomes indeed often have a detrimental effect (*cf.* Müntzing, 1943 ; Randolph, 1941) and particularly on the seed fertility. In *T. grandiflorum* this is, however, not the case. The seed setting of crosses between plants without fragment chromosomes is smaller than in crosses between fragment plants (Rutishauser, 1956). A further result is also striking : the effect of the fragment chromosomes is not noticeably cumulative. The breakage frequency is not significantly increased by the introduction of more than one fragment chromosome. This result, too, supports the conclusion that a special influence is exerted by the fragment chromosome and that the spontaneous chromosome breakages in the endosperms of *T. grandiflorum* are genetically controlled.

The assumption that spontaneous chromosome breakages are subjected to a genetic control is supported by the results of experiments on the somatic mutability of maize (Rhoades, 1936, 1938) and of some species of cotton (Harland, 1937). In both plant groups genes could be found which significantly raised the mutation rate. The mutator gene of *Zea mays* (Dt) is in addition active in the endosperm and there

it influences the mutability of a colour factor. Unlike spontaneous chromosome breakage it is here of course a matter of gene and not chromosome mutation. Rhoades considers it probable that chemical substances are produced by the Dt-gene which raise the mutation rate. The results of my own experiments also lead to a similar view.

Spontaneous chromosome breakages have much in common with chemically induced breakages, as has been pointed out particularly by Gray (1952). According to Revell (1952) the latter differ from breakages produced by ionising rays in that they are concentrated in certain places in the chromosome and that certain chromosomes are affected more readily than others. The *Trillium* experiments point in the same direction : the distribution of chromosome breaks is not a chance one. The E-chromosome is preferred, and the C-chromosome rarely breaks. The distribution of breaks over the different regions does, however, seems to be more or less haphazard. Only in the D-chromosomes is the heterochromatic region more frequently broken than the euchromatic. More marked is the unevenness of distribution of chromosome breaks in hybrid endosperms of the cross *Paris quadrifolius* × *T. grandiflorum* (Rutishauser and La Cour, 1956). There, for example, in the E-chromosome twelve times as many breaks occur as in the B-chromosome. The view mentioned several times above that the chromosome breaks are caused by a disturbance of the cell metabolism, gains another support by these considerations.

Brock (1954, 1955) expounds the view that the seed-fertility is also influenced by errors in division in his work on spontaneous chromosome breakages in the endosperm of lily hybrids and hyacinths. The formation of persisting bridges is regarded as the immediate cause of this. They lead to the development of polyploid nuclei and so to a disturbance of the chromosome balance.

In *Trillium* endosperms SR and consequently bridges are relatively rare. The centric fragments are usually passed on unchanged. McClintock's breakage-fusion-bridge cycle does not come into action, at least in older endosperms, or else it is not compulsory. Similar observations have been made by Brock himself in connection with hyacinths. With *T. grandiflorum* also no snowball spreading of chromosome breakages can be shown. It is true that the distribution of breaks over the 2207 analysed mitoses is not a chance one. But the breakage frequency seems to be only slightly influenced by previous chromosome breakages.

Polyploid and spontaneous chromosome breakage do not reduce the seed fertility of *T. grandiflorum*. As already mentioned, the seed setting of the crosses with fragment plants tends to be bigger than that of plants which have none. So it appears that it is at least debatable whether in this species spontaneous chromosome breakage can be regarded as the cause of the reduced seed setting. In a further work it will be shown that the failure of endosperm development in

Trillium × *Paris* hybrids is not connected with an increase in spontaneous breakage frequency.

7. SUMMARY

1. The staining of heterochromatic segments (H-segments) in *T. grandiflorum* depends not only on the temperature, but also on the functional condition of the tissue. As a rule weaker stained nuclei reveal weaker stained H-segments. Their coiling processes are also affected.

2. The H-segments of *T. grandiflorum* are with few exceptions constant in size, number and position in the same individual, but they vary from individual to individual in the same batch of samples. The E-chromosome varies most. The aberrant chromosomes mostly occur in heterozygous combinations, as a rule only standard types are homozygous.

3. The 5 chromosome types are as a rule passed on to the endosperms unchanged. By non-disjunction 1 per cent. of female and male gametes develop with hyperhaploid and hypohaploid chromosome complements. It is greater on the male than on the female side.

4. The following properties of *T. grandiflorum* permit a cytological investigation of the consequences of crossing-over in the endosperm: (*a*) the homologous chromosome pairs are occasionally heteromorphic. (*b*) The two chromatids which are separated in the second meiosis are brought together again in the endosperm, so that it is possible to carry out " tetrad-analyses ".

5. The number of post-reductions varies between 56 and 84 per cent. for B-, C- and E-chromosomes. The frequency of post-reductions is not proportional to the measured distance between the centromere and the point where the difference begins. This finding is due to the localisation of the chiasmata.

6. In the endosperms of *T. grandiflorum* centric fragments, rings, dicentric and telocentric chromosomes appear as consequences of spontaneous chromosome breakage with a frequency of 1·06 per cent. The frequency of initial breakage is reckoned at 0·80 per cent. The centric fragments are passed on unchanged for the most part.

7. The breakages are distributed unevenly over the 5 *Trillium* chromosomes. The E-chromosome is most breakable, the C-chromosome least.

8. The frequency of spontaneous chromosome breakage is dependent on tissue. In root cells no aberrant chromosomes are found.

9. Fragment chromosomes (B- or accessory chromosomes) raise the breakage frequency. From this it is concluded that the spontaneous chromosome breakage is controlled genetically.

10. The development of the endosperm is not influenced unfavourably by spontaneous chromosome breakages.

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GENOTYPIC CONTROL OF CHROMOSOME BEHAVIOUR IN RYE. III. CHIASMA FREQUENCY IN HOMOZYGOTES AND HETEROZYGOTES

H. REES and J. B. THOMPSON

Agricultural Research Council Unit of Biometrical Genetics, Department of Genetics, University of Birmingham

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1. INTRODUCTION

EARLIER work has shown that the average chiasma frequencies found in p.m.c. of rye plants are subject to the control of the genotype (Rees, 1955*a* and *b*). It has also been established that the control is polygenic, except in rare cases where a single recessive gene may cause asynapsis (Prakken, 1943). Something of the nature of this polygenic control becomes evident from the results of inbreeding experiments which have been carried out in this species (Lamm, 1936 ; Müntzing and Akdik, 1948). Normal rye populations outbreed and hence comprise genotypes which are partially heterozygous. It was found that inbreeding led to a reduction in chiasma frequency, and often to failure of metaphase pairing. Efficient chiasma formation thus depends upon a genetic balance achieved through polygenes in partially heterozygous combinations. The experiment to be described was carried out to discover how such a system of genes operates. For this purpose a sample of homozygous inbred lines were crossed in a number of combinations and the results from parent lines and their F_1s have been analysed using, as far as possible, the techniques available for investigating continuous variation.

We are concerned with the effects of crossing homozygotes on two different aspects of the variation in chiasma frequency. In the first place we need to discover in what manner this affects the mean frequency of chiasmata in p.m.c. of different genotypes. In the second place we can investigate the environmental, non-heritable, variation in the chiasma frequency of p.m.c., and in particular we can compare the extent of such variation in homozygotes and heterozygotes. For studying this non-heritable component of variation chiasma frequency is a particularly useful character, because the variation can be measured at three levels (i) between plants of the same genotype. (ii) between p.m.c. within plants, and (iii) between bivalents within p.m.c. From such investigations we can hope to gain a better understanding of the genetic and physiological interrelations which govern the expression of chiasma frequency as a property of the nuclear phenotypes at meiosis.

2. MATERIAL AND METHOD

Chiasma frequency was scored in 20 p.m.c. in each plant, and from this scoring three metrics were derived :—

1. The plant chiasma frequency, which is expressed as the *average per bivalent* (*cf.* Rees, *l.c.*) of the 20 cells scored.

2. The cell variance, which is the variance—with 19 degrees of freedom—between the p.m.c. in chiasma frequency per bivalent.
3. The bivalent variance, being the variance in chiasma frequency—with 120 degrees of freedom (there being seven bivalents, and therefore six comparisons, in each of the 20 p.m.c.)—between bivalents within p.m.c.

TABLE 1

Plant chiasma frequencies, cell variances and bivalent variances in the inbred lines grown in 1954

	P3								
Plant	1	2	3	4	5	6	7	8	9
Chiasma frequency	1·864	1·886	1·836	1·871	1·857	1·793	1·843	1·886	1·850
Cell variance	0·067	0·099	0·124	0·179	0·105	0·128	0·209	0·159	0·203
Bivalent variance	0·209	0·186	0·157	0·136	0·193	0·188	0·205	0·193	0·183

	P13								
Plant	1	2	3	4	5	6	7	8	9
Chiasma frequency	1·621	1·750	1·529	1·550	1·807	1·850	1·729	1·871	1·821
Cell variance	0·320	0·359	0·332	0·365	0·260	0·203	0·224	0·104	0·209
Cell bivalent	0·290	0·245	0·338	0·431	0·174	0·200	0·212	0·181	0·205

	P6								
Plant	1	2	3	4	5	6	7	8	9
Chiasma frequency	1·714	1·650	1·771	1·586	1·650	1·764	1·729	1·729	1·800
Cell variance	0·091	0·158	0·126	0·856	0·789	0·200	0·209	0·164	0·171
Cell bivalent	0·224	0·291	0·219	0·264	0·224	0·195	0·264	0·271	0·176

	P12									
Plant	1	2	3	4	5	6	7	8	9	10
Chiasma frequency	1·650	1·743	1·750	1·764	1·607	1·700	1·743	1·593	1·693	1·771
Cell variance	0·353	0·174	0·224	0·365	0·224	0·239	0·129	0·260	0·200	0·096
Cell bivalent	0·332	0·245	0·250	0·254	0·343	0·374	0·286	0·340	0·217	0·207

All means and variances for convenience are expressed in terms of the chiasma frequency per bivalent.

The lines have been inbred by self pollination for more than 20 generations and are assumed to be virtually homozygous. The four lines used in the diallel experiment to be described were crossed in all combinations to produce six F_1 families. Because of shortage the seed from reciprocal crosses was pooled and the plants scored without reference to the way in which the cross was made.

3. HETEROSIS

The chiasma frequency distributions in the parent lines and their F_1s are shown in tables 1 and 2 and in fig. 1. We may note in respect of the mean values of lines and F_1s the following features :—

(1) The lines differ from one another (P = <0·01).

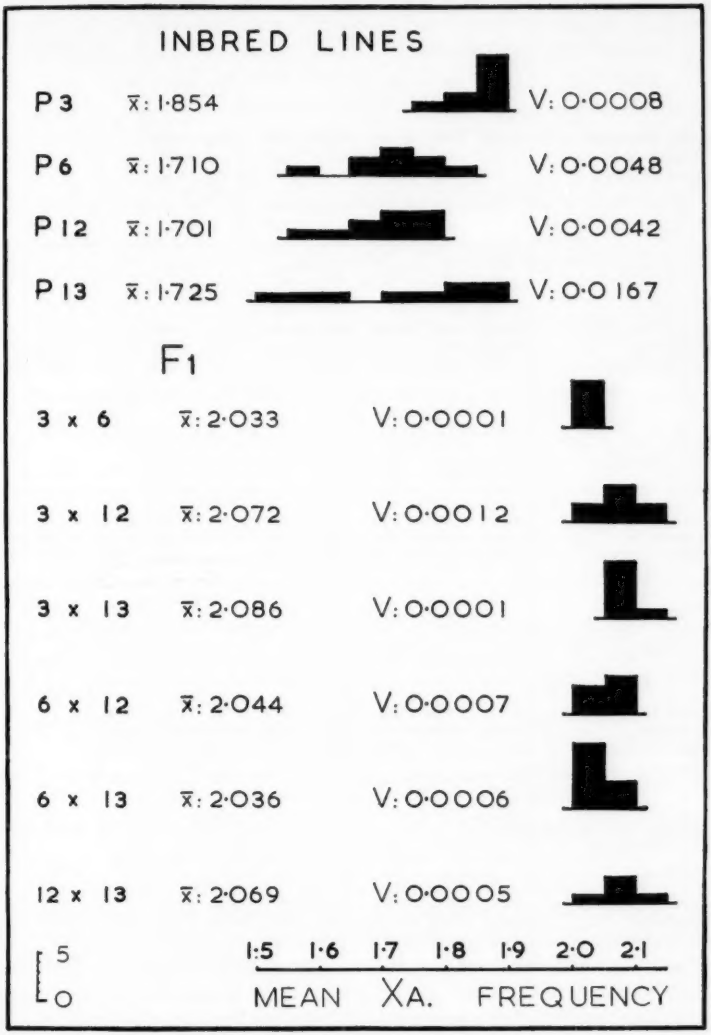

FIG. 1.—The distributions of plant chiasma frequencies in four inbred lines and their F_1s. The F_1 families have higher means than the inbred lines. Means ($\bar{x}$) and variances (V) are listed. The vertical scale for the histograms is at the bottom, left. Xa= chiasma.

(2) All F_1s have higher means than the inbred lines ($P = <0{\cdot}001$).
(3) The F_1s differ from one another ($P = <0{\cdot}001$).

TABLE 2

Plant chiasma frequencies, cell variances and bivalent variances in the F_1 families grown in 1954

	3×13						
Plant	1	2	3	4	5	6	7
Chiasma frequency	2·093	2·071	2·107	2·086	2·086	2·079	2·086
Cell variance	0·109	0·038	0·073	0·111	0·051	0·052	0·081
Bivalent variance	0·081	0·138	0·217	0·140	0·100	0·093	0·095

	6×13									
Plant	1	2	3	4	5	6	7	8	9	10
Chiasma frequency	2·043	2·029	2·021	2·007	2·021	2·079	2·029	2·071	2·007	2·050
Cell variance	0·047	0·084	0·064	0·037	0·094	0·067	0·084	0·083	0·097	0·245
Bivalent variance	0·057	0·086	0·097	0·052	0·110	0·091	0·086	0·114	0·068	0·167

	3×6					12×13				
Plant	1	2	3	4	5	1	2	3	4	5
Chiasma frequency	2·043	2·036	2·043	2·014	2·029	2·071	2·050	2·100	2·043	2·079
Cell variance	0·122	0·118	0·167	0·104	0·144	0·218	0·094	0·122	0·092	0·097
Bivalent variance	0·145	0·138	0·105	0·167	0·109	0·143	0·090	0·169	0·200	0·102

	3×12							
Plant	1	2	3	4	5	6	7	8
Chiasma frequency	2·107	2·086	2·064	2·129	2·071	2·021	2·057	2·043
Cell variance	0·133	0·262	0·127	0·119	0·143	0·185	0·081	0·137
Bivalent variance	0·224	0·200	0·183	0·162	0·155	0·179	0·100	0·159

	6×12						
Plant	1	2	3	4	5	6	7
Chiasma frequency	2·007	2·036	2·086	2·050	2·021	2·050	2·057
Cell variance	0·143	0·164	0·217	0·185	0·125	0·109	0·081
Bivalent variance	0·102	0·148	0·191	0·143	0·071	0·105	0·083

(1) evidently reflects genotypic differences between the lines which have been noted elsewhere (Rees, *l.c.*) and which need not be discussed further here.

(2) and (3) we need to consider in some detail.

The higher values in heterozygous F_1s clearly demonstrate heterosis for chiasma frequency. Since inbreeding in rye reduces the chiasma frequency and crossing inbred lines restores the higher values, it follows, as already pointed out, that in the F_1 hybrids and in population plants the high average chiasma frequency must depend on genetic balance in heterozygous genotypes. It will be seen, however, that, although all the F_1s exhibit heterosis, particular heterozygotes express heterosis to different degrees: not all the heterozygous combinations are

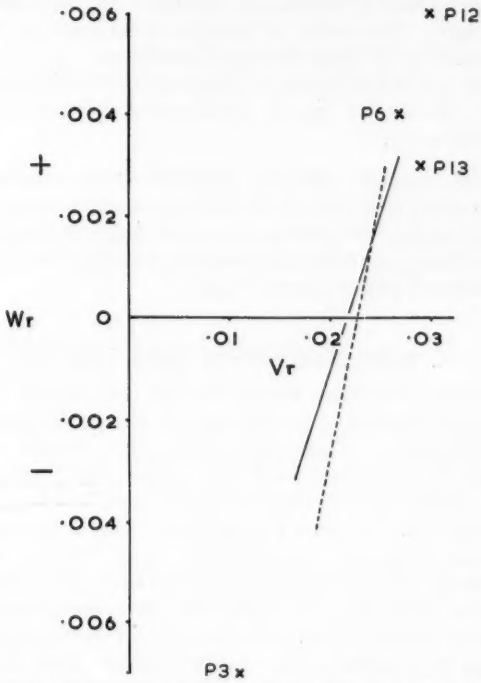

FIG. 2.—The covariance-variance graph of chiasma frequency in the diallel cross. The dotted line represents a slope of 1. Explanation in text.

balanced to the same extent either in the F_1s above, or in population plants (*cf.* Müntzing and Prakken, 1941).

The average efficiency of the population heterozygotes will evidently have been achieved under the influence of natural selection. We need now to inquire by what kind of gene interactions, allelic or non-allelic, such an efficiency is achieved and maintained. It is an important consideration because the answer would tell us how genetic recombination, through chiasma frequency, may be regulated in normal populations of outbreeding individuals.

Recently a method for detecting allelic and non-allelic interaction in diallel crosses of the kind we are dealing with has been developed

by Jinks (1954) and Hayman (1954). The interactions are identified by plotting the variance of the offspring means (Vr) against the covariance (Wr) of the offspring family means with the means of the non-recurrent parents. This has been done with our data in fig. 2. The basis of interpreting the graph is described fully by the authors in the papers referred to above. The conclusions to be drawn from the graph are briefly :—

(i) There is high average " overdominance ", since the regression line, which is significant, intersects the Wr axis below the origin. The order of average dominance is P3, P13, P6 and P12, P3 being the most dominant.

(ii) There is evidence of non-allelic interaction because the slope of the Wr/Vr line is significantly different from 1 ($P = 0{\cdot}05$-$0{\cdot}02$).

It appears therefore that the apparent overdominance in mean chiasma frequency is at least partly due to non-allelic gene interaction, as indeed we might have expected on the basis of Jinks' analyses of heterosis involving a number of characters showing " overdominance " in a wide range of species (Jinks, 1955).

4. VARIATION WITHIN LINES AND F_1s

The chiasma variation within the F_1s and within the lines is between similar genotypes, so that we are here concerned with non-heritable differences. These differences arise through variation in uncontrolled environmental conditions under which the plants grow, though just what these variable conditions are is not easy to ascertain with any certainty. Weather changes from day to day are known to affect chiasma frequency (*e.g.* Mather, 1935), but in this instance such changes are of small importance, the effect of fixing on different days being insignificant. It is probable that the growth history of the plant over a long period may be reflected in the activities of the chromosomes at meiosis. There is for instance some evidence from another experiment that growing rye plants in different parts of the field may affect the chiasma frequency (Thompson, unpub.).

Whatever the factors causing the variation there is no doubt, as has been recently pointed out (Rees, 1955*b*) that the amount of variation between plants measured as the variance is much greater in the homozygous lines than in the heterozygous F_1s (fig. 1). This we have interpreted as reflecting the greater developmental stability, or homeostasis (see Lerner, 1954) of the heterozygotes in respect of chiasma frequencies.

Comparable proof of the greater stability of the heterozygotes is obtained when we consider the variation between cells within F_1 plants, and within inbred plants. The average variances are presented in table 3. Similarly when we consider the variation between bivalents

within p.m.c. (table 4). Both between p.m.c. and within p.m.c. the variation is significantly greater (P = <0·001) in the homozygotes.

While the distinction between heterozygotes and homozygotes is clear enough, high means and low variances, low means and high

TABLE 3

The average cell variances in the inbred lines and F_1 families

	P3	P6	P12	P13
P3	*0·141*	0·131	0·148	0·074
P6		*0·307*	0·146	0·090
P12			*0·226*	0·125
P13				*0·264*

variances respectively, there now arises the problem of the extent to which these variables are dependent upon each other. For example, does the degree of stability as measured by chiasma variation under a given range of growing conditions depend directly on the amount of

TABLE 4

The average bivalent variances in the inbred lines and the F_1 families

	P3	P6	P12	P13
P3	*0·183*	0·133	0·170	0·123
P6		*0·256*	0·120	0·093
P12			*0·285*	0·141
P13				*0·253*

heterozygosity of the genotype? Is it dependent to any extent on the mean chiasma frequency? We shall try to answer such questions in the following sections. At the same time it will be necessary to attempt to explain in physiological and genetical terms the basis for the variation between nuclei of identical genotypes.

(i) *Plant variation*

The variation in chiasma frequency between plants, as we have seen, is greater within the inbred lines than F_1s. There are, however, considerable and highly significant differences between the amounts of variation in different lines. The Bartlett test of homogeneity of the variances, which are listed in fig. 1, gives a $\chi^2_{[3]} = 15{\cdot}571$ (P = 0·01-0·001). Evidently this reflects genotypic diversity in respect of the stability properties of the inbred lines. Since the inbred lines are, virtually, homozygous this diversity cannot be related to varying degrees of hybridity. Neither are the line variances correlated, to

any appreciable extent at least, with the mean chiasma frequencies : the regression of line means and line variances is not, in fact, significant (see below).

Item	SS	N	MS	VR	P
Regression . .	0·030197	1	0·030197	<1·0	Not significant
Error . . .	0·123103	2	0·061551	...	...
Total . .	0·153300	3	...	...	...

These observations have been confirmed in the data from the five inbred lines which were scored in 1952 (see Rees, 1955*a*). The data are given in table 5. Four of these lines were each represented by a pair of sub lines. The Bartlett test showed a significant difference in variances between lines ($\chi^2_{[4]} = 9{\cdot}641$, $P = 0{\cdot}05\text{-}0{\cdot}02$) and no significant difference within lines, *i.e.* between sub lines ($\chi^2_{[4]} = 2{\cdot}308$, $P = 0{\cdot}70\text{-}0{\cdot}50$). The regression between line means and line variances, as for the 1954 data, is not significant. The between plant variances for the six F_1 families are not significantly different.

Taking into account therefore the data from the two years it would appear that stability in the inbred lines, measured as the variation in chiasma frequency between plants, is determined by the particular homozygous genotypic combination. At the same time, while some homozygous genotypes show greater stability than others, generally they show less stability than the heterozygous F_1s. Finally we have shown that this variation is, at least largely, independent of the mean chiasma frequency.

(ii) *Cell variation*

The distributions of cell variances (1954 data) are significantly different between inbred lines ($P = <0{\cdot}001$). As for plant variation, it therefore appears that the amount of variation between p.m.c. in an anther is subject to the control of the genotype, and can vary between genotypes of equal hybridity.

This variation between the p.m.c. within a plant is not independent of the chiasma frequency. *Within lines* there is a strong and significant negative correlation between them (see table 1). Below is the joint regression analysis of variance which enables us to compare the

Item	SS	N	MS	VR	P
Joint regression . . .	0·5308	1	0·5308	14·336	<0·001
Heterogeneity of regressions .	0·5610	3	0·1870	5·051	0·01-0·001
Heterogeneity of line means (Xta)	1·4005	3	0·4668	12·609	<0·001
Error	1·0737	29	0·0370	...	...
Total	3·5660	36	...	...	...

relationships between chiasma frequencies and cell variances in the four lines. The joint regression item is highly significant. It will

TABLE 5

Plant chiasma frequencies, cell variances and bivalent variances in five inbred lines grown in 1952. Four of the lines are each represented by a pair of sub-lines

	P2				
Plant	1	2	3	4	5
Chiasma frequency	2·043	2·079	2·043	2·014	1·786
Cell variance	0·393	0·263	0·332	0·074	0·459
Bivalent variance	0·269	0·360	0·312	0·338	0·271

	P4					P5				
Plant	1	2	3	4	5	1	2	3	4	5
Chiasma frequency	1·736	1·571	1·700	1·450	1·786	1·414	1·764	1·543	1·686	1·736
Cell variance	0·500	0·571	0·329	0·485	0·218	1·352	0·260	0·686	0·445	0·500
Bivalent variance	0·348	0·445	0·326	0·362	0·312	0·402	0·077	0·381	0·264	0·281

	P7					P8				
Plant	1	2	3	4	5	1	2	3	4	5
Chiasma frequency	1·764	1·764	1·779	1·743	1·871	1·643	1·721	1·671	1·829	1·729
Cell variance	0·455	0·230	0·413	0·490	0·224	0·429	0·458	0·693	0·250	0·420
Bivalent variance	0·455	0·474	0·436	0·529	0·329	0·533	0·429	0·464	0·376	0·364

	P13					P14				
Plant	1	2	3	4	5	1	2	3	4	5
Chiasma frequency	1·900	1·979	1·914	1·879	1·971	1·864	1·900	2·036	1·850	1·957
Cell variance	0·408	0·455	0·217	0·470	0·220	1·398	0·393	0·254	0·443	0·317
Bivalent variance	0·357	0·419	0·374	0·450	0·431	0·357	0·276	0·250	0·379	0·314

	P17					P18				
Plant	1	3	4	5	8	1	2	3	5	8
Chiasma frequency	1·679	1·571	1·636	1·714	1·664	1·521	1·664	1·629	1·643	1·750
Cell variance	0·284	0·316	0·248	0·301	0·455	0·320	0·290	0·232	0·278	0·404
Bivalent variance	0·310	0·336	0·331	0·391	0·338	0·391	0·314	0·319	0·291	0·305

also be observed that the sum of squares for heterogeneity is significant, which argues that although the lines show the same general physiological relationship between cell variation and chiasma frequency,

the rate of change of one relative to the other is not the same for every line. This may justifiably be attributed to genotypic causes.

Analysis of the 1952 data gives the same results except that the sum of squares for heterogeneity of regressions was not quite significant ($P = 0{\cdot}2\text{-}0{\cdot}05$).

The negative correlation between cell variances and plant chiasma frequencies within families suggests the possibility of a causal relationship between the two properties. We have in fact obtained good evidence for such a relationship from an investigation of the chiasma distributions of large numbers of p.m.c. of inbred plants. The

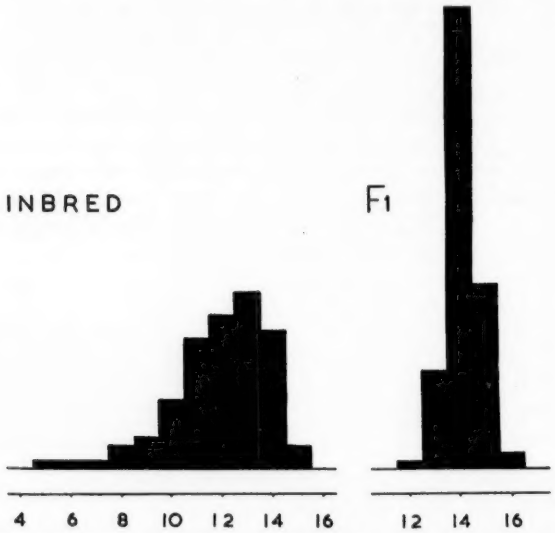

FIG. 3.—The distributions of chiasma frequencies in 100 p.m.c. in an inbred plant and in an F_1 plant. In the F_1 the mean is higher and the spread smaller. In the inbred plant the distribution is significantly skew. Chiasmata per cell on the horizontal scales.

distribution curves of chiasma frequencies of 100 p.m.c. in each of nine plants from three lines were plotted. In general these curves show (1) a longer tail towards the lower values, and (2) a steep slope at the higher end (fig. 3). In three of the plants the skewness of the distributions is highly significant ($P = <0{\cdot}001$).

These curves indicate a stricter limitation to the development of cells with high rather than with low frequencies. There is an upper limit in the number of chiasmata per cell (*cf.* Mather, 1936). From these observations it would follow that an increase in variation arising within an anther is more likely to be in the direction of cells with fewer chiasmata ; and so the greater the variation between cells the lower the plant chiasma frequency. In these inbred lines therefore a high degree of stability in development, as measured by cell variances

results in a higher mean, and *vice versa*. We do not suggest that the amount of cell variation is the only factor affecting the plant chiasma frequency. Thus the regressions between the mean chiasma frequencies of lines and the mean cell variances of lines are not significant, either for the 1954 or 1952 data.

The relation found here between the degree of stability of body parts and mean values of character expression is not of course universal (*cf.* asymmetry in flies, Mather, 1953). It depends in this instance on the upper limit imposed upon the expression of chiasma frequencies in the p.m.c.

In the F_1 families the chief difference from the inbred lines is that the cell variances are smaller (table 3, fig. 3). As in the inbred lines (1) there are significant differences between families ($P = 0{\cdot}05$-$0{\cdot}01$), (2) there is no significant regression between the mean cell variances of families and the mean chiasma frequencies of families. In contrast to the inbred lines, however, we find the regression between cell variances and chiasma frequencies within families is not significant.

When we seek to explain the material basis for the variation between p.m.c. within an anther we can, as pointed out earlier, rule out variation in nuclear genotypes within it, since the nuclei must be genetically identical. The variation must result from differences arising outside the nuclei, in the cytoplasm, which affect their behaviour. These cytoplasmic differences are initiated no doubt by local environmental fluctuations within the anther. In those anthers where the cytoplasm is more resistant to qualitative changes, and therefore more uniform, under a given set of growing conditions the p.m.c. variation will be low and the average chiasma frequency consequently relatively high. Furthermore we conclude that since cell variation varies between lines and families the *extent* of this variation arising in the cytoplasm is under the influence of the genotype.

(iii) *Bivalent variation*

The distributions of bivalent variances within cells again are significantly different between the lines ($P = <0{\cdot}001$). *Within lines* bivalent variances, like cell variances, are negatively correlated with the chiasma frequencies ($P = <0{\cdot}001$). *Between lines* the regression between average variances and average chiasma frequencies is not significant. In the F_1s also the situation is much the same as for cell variances, (1) the bivalent variances are smaller (table 3), (2) the families show significant differences ($P = 0{\cdot}01$-$0{\cdot}001$), and (3) the regressions between chiasma frequencies and bivalent variances are not significant either within families or between family averages. It will be seen that differences in bivalent variances between genotypes (*e.g.* of inbred lines) can, like differences in cell variances, be independent of the degree of hybridity.

Many factors are known to affect variation in chiasma frequency between bivalents within p.m.c. (*cf.* Darlington, 1937). These

depend upon (1) genetic (including structural) differences between chromosome pairs, and (2) the cytoplasmic environment of p.m.c. nuclei before or during the early stages of meiosis. The latter may be modified by the genotype or by external conditions. A likely explanation for the genotypic differences in the present experiment has been suggested to us by Professor Darlington, *viz.* that the pre-pachytene pairing arrangement of homologous chromosomes within the nucleus may be less regular in some genotypes than in others. Such irregularity might even begin as early as the pre-meiotic telophase. Most probably the regularity in chromosome arrangement would depend on the cytoplasmic environment within the cell, and this would vary between genotypes. On this view arrangement would, in general, be irregular in homozygotes, regular in heterozygotes.

It was pointed out earlier that bivalent variances and chiasma frequencies are negatively correlated between plants within inbred lines. This has been shown in another way. Regressions were calculated between the twenty p.m.c. bivalent variances and chiasma frequencies scored within single anthers. The mean regression for eight inbred plants (two at random from each line) is significant ($P = <0{\cdot}001$). This relation between the chiasma frequency and the bivalent variation in inbred lines is not surprising if, as suggested above, bivalent variation in the inbred plants results from irregularity in pairing arrangement: the more irregular the pairing the lower we should expect the chiasma frequency to be. Earlier it was postulated that chiasma variation between p.m.c. within an anther was due to cytoplasmic variation between the cells. It may reasonably be assumed this operates at least to some extent by influencing the pairing arrangements of the chromosomes.

The relation between bivalent variation and p.m.c. chiasma frequency is, however, by no means simple and direct. We find, for example, an entirely different situation *within anthers* of F_1 plants where regression analyses demonstrate a significant positive correlation between bivalent variances and p.m.c. chiasma frequencies. If pairing arrangements in F_1 p.m.c. are regular, other factors, such as genetic differences between bivalents, would be more clearly revealed, and may well be responsible for the variation.

5. DIALLEL ANALYSES INVOLVING THE VARIANCES

By comparing lines and families we have shown that developmental stability in respect of chiasmata, in whichever of the three ways we measured it, is strongly subject to genotypic control. An important question which then arises is whether stability measured at these three different levels is controlled by the same gene system. It is possible to gain information on this point by plotting Wr/Vr graphs using the variances as our metrics. A comparison of these graphs will then show whether the inheritance is the same for each variance. The graphs are shown in fig. 4.

For plant variances the regression line, which is significant (P = 0·01-0·001) passes near to the origin and its slope is not significantly different from 1. There is thus no evidence for non-allelic interaction or for " overdominance ". The order of average dominance is P12, P3, P6, P13.

The Wr/Vr regression line for cell variances, which is also significant, passes below the point of origin, indicating " overdominance ". The slope ($b = 0{\cdot}558$) is significantly different from 1 (P = 0·02-0·01),

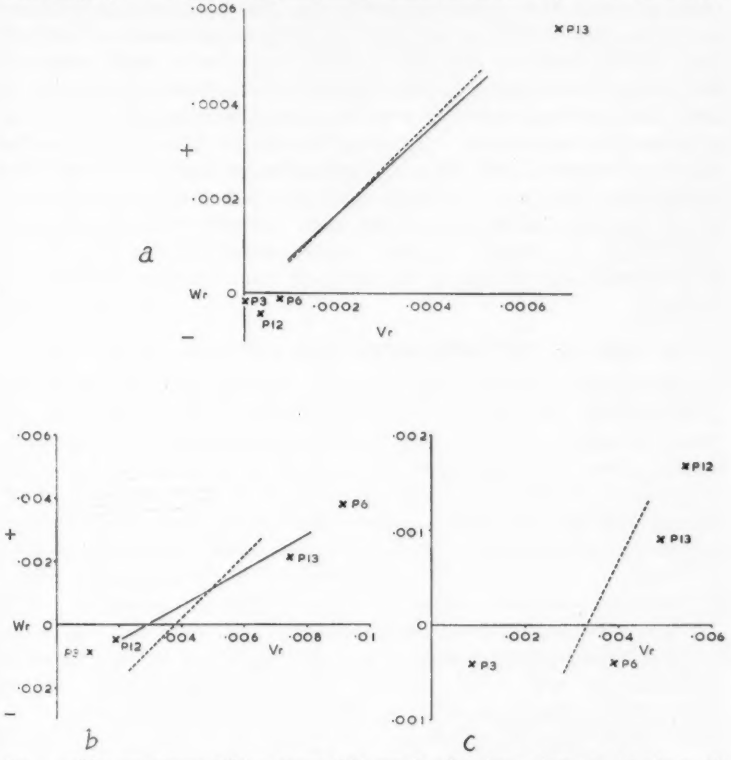

FIG. 4.—The covariance (Wr)-variance (Vr) graphs of variances between (*a*) plants, (*b*) cells and (*c*) bivalents in the diallel cross. The dotted line in each case represents a slope of 1, the solid line the Wr-Vr regression. Explanation in text.

and therefore is evidence for non-allelic interaction. In both these respects the inheritance of cell variances is different from plant variances. In addition, the order of dominance is not the same, being P3, P12, P13, P6.

With regard to bivalent variances the Wr/Vr line is not significant. This possibly may be accounted for by strong interaction in certain genotypes. Other causes, however, may be responsible and no definite conclusions can be drawn from the graph in respect of the inheritance

of this property. We may note, however, that the order of dominance distinguishes it from the other two (P3, P6, P13, P12).

It is evident from these facts that chiasma variation at one level is not controlled in a way identical to that exercised at other levels. Indeed our evidence strongly indicates the control to be at least partly different for each variance.

It will also be seen that these graphs differ from that for mean chiasma frequency (fig. 2), either in slope or dominance relationships. This supports the earlier evidence, *viz.* the absence of significant regressions between mean chiasma frequencies and variances of inbred lines and F_1 families, that the variances can be at least partly independent of the mean in inheritance. The independence of mean and variances in inheritance is of course not incompatible with their physiological correlation. For example, it has been shown earlier that cell variances and chiasma frequencies are negatively correlated within lines by virtue of a more or less well defined " upper limit " to the number of chiasmata per cell. Despite this physiological relationship the mean can vary independently of the variance in different genotypes where, for instance, the " upper limits " are different.

6. GENETIC RECOMBINATION AND NATURAL SELECTION

In chiasma frequency the two main features that distinguish rye heterozygous genotypes from homozygotes are (1) the average per plant is higher, and (2) the variation between cells and bivalents within plants (*cf.* Lamm, 1936), and between plants of the same genotype, is smaller. The smaller variation in heterozygotes, within plants, and between plants of the same family, both reflect greater stability in development. It has been shown that, although this kind of variation is initiated by environmental fluctuations, the *amount* of variation exhibited depends on the particular genotype. Now heterozygous genotypes in general show less variation, *i.e.* show greater stability, than homozygotes. But it cannot be the degree of hybridity in itself which directly determines this, because inbred lines equally homozygous show different degrees of stability. This view agrees with the conclusions of Jinks and Mather (1955) based on evidence from *Nicotiana rustica*, a partial inbreeder, and from *Drosophila melanogaster* (Mather, 1954). It appears therefore, as these authors point out, that the high degree of developmental stability (associated in this case with rye heterozygotes) has been achieved by the action of natural selection for particular, balanced, genic combinations.

There is no doubt that plant chiasma frequencies, and hence the *average* amount of genetic recombination in a population is adjusted by natural selection, and thereby controls the release of variability in that population. We can now also see how the *range* of recombination values of gametes produced by the same genotype may be adjusted,

and this to some extent at least independently of the mean frequency. First, the variation between p.m.c. within a plant can be controlled. Secondly, there can be control of the manner in which chiasmata are distributed between the bivalents within p.m.c. such that the chiasmata are more, or less, equally shared by the bivalents. Both adjustments within the anther would affect the *variety* of gametes produced. The mechanism of this control, we have suggested, acts *via* the cytoplasm. Since the amount of cell and bivalent variation is relatively small in heterozygotes, we conclude that a relative uniformity between gametes in respect of recombination is an adaptive feature of rye populations.

It must be noted that although we may regard the large degree of environmentally induced variation between plants, cells and bivalents in inbred genotypes as reflecting developmental instability, the very mechanism of this instability can be controlled in such a way as to regulate the recombination properties of a population. We may sum up by saying that genetic recombination is subject to the control of the genotype at three levels, the plant, the cell and the bivalent, at any of which the values are capable of adjustment by natural selection.

7. SUMMARY

1. Four inbred lines of rye were crossed in all combinations and the chiasma frequency was scored in the parent lines and the six F_1 families. The average chiasma frequencies are higher in the F_1 heterozygotes than in the homozygous lines : the F_1s exhibit heterosis for chiasma frequency.

2. The inheritance of plant chiasma frequency shows apparent " overdominance " in F_1. This is in part at least explained by non-allelic interaction.

3. Analysis of environmentally induced variation, measured by comparisons within identical genotypes, gave the following results :

(1) There is less variation between plants within F_1 families than within inbred lines.

(2) There is less variation between p.m.c. and between bivalents within p.m.c. in heterozygotes than in homozygotes.

Thus, in respect of chiasma frequencies, under the conditions of the experiment the heterozygous genotypes show greater stability in development than the homozygotes.

4. Inbred lines equally homozygous differ in stability. Stability therefore is not simply related to the degree of hybridity of the genotype.

5. Stability, measured as the variation between plants, between cells and between bivalents is genotypically controlled ; but the control is not the same for the variation shown at these three levels. T he gene system controlling the mean plant chiasma frequency is also

different, so that variances and means, to some extent at least, can show independence in inheritance.

6. It is postulated that variation in chiasma frequency between p.m.c. results from intercellular variation in the cytoplasm which influences chiasma formation. This cytoplasmic variation is much less in heterozygotes than in homozygotes. It also varies between inbred lines.

7. *Within* inbred lines chiasma frequencies are negatively correlated with cell variances. On our view this means that within an inbred line cytoplasmic variation between cells is greatest in plants with the lower chiasma frequencies.

8. It is also demonstrated that the chiasma frequency per cell in an inbred plant has an upper limit. An increase in cell variation therefore lowers the mean, and this explains the negative correlation.

9. Bivalent variances, like cell variances, are negatively correlated with chiasma frequencies within inbred lines. The bivalent variation and the correlation with chiasma frequency may, in part, be explained in terms of irregularity of pre-pachytene pairing.

10. It is concluded that selective adjustment in chiasma frequency and hence in genetic recombination is possible at the three levels of plant, cell and bivalent.

Acknowledgments.—We are indebted to Professor K. Mather, Dr J. L. Jinks and Dr B. I. Hayman of this Unit, and to Professor C. D. Darlington, for their advice and criticism.

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THE BLOOD GROUPS AND HÆMOGLOBINS OF THE SIKHS

G. W. G. BIRD, ELIZABETH W. IKIN, H. LEHMANN and A. E. MOURANT
Lister Institute, London, S.W. 1

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BLOOD-GROUP studies show, beneath the diversity of the settled peoples of India, an underlying uniformity, whereas many of the aboriginal tribes differ from these people in a more marked and fundamental manner. The numerous and scattered references to these studies have been listed by Boyd (1939) and Mourant (1954) who have also collected most of the published data into tables.

The settled peoples show varying but always high frequencies of group B, and rather high frequencies of A. The aboriginal tribes may or may not show these features, and some show ABO frequencies which in different ways are near the extreme limits found among human populations. The only blood-group system other than the ABO, for which the data are adequate to show systematic differences within the Indian sub-continent, is the Rh system. With respect to the latter, most of the settled peoples so far sampled show frequencies closely similar to those of the Mediterranean peoples who have, however, very different ABO frequencies. There are usually 5 to 10 per cent. of Rh-negatives (mostly cde/cde), with a very high frequency of genotypes containing CDe (R_1), and with cDE (R_2) present but less frequent than in northern Europe. The chromosome CDE (R_z) is absent or very rare.

Many of the aboriginal tribes, on the other hand, are entirely lacking in Rh-negatives and among them CDE is not very rare. Some of these tribes, of a Veddoid type, and living in the Nilgiri Hills, have a moderately high frequency of the sickle-cell trait which appears to be absent from the settled peoples of the same region (Lehmann and Cutbush, 1952). The settled peoples in general are, however, far from being genetically uniform—they are apparently less so, for example, than the peoples of northern and central Europe. This is particularly shown in the study by Sanghvi and Khanolkar (1950-51) of a number of different castes in Bombay. There is, therefore, still considerable scope for blood-group studies of different communities; especially if these tend to be exclusively endogamous. In order, however, to discover statistically significant differences between the communities, it will become necessary to test larger numbers of persons than have usually been examined in the past, and any considerable extension of the work must depend on the development of laboratory facilities and serum supplies in India.

In the present paper we describe an investigation which was in

the first place intended to cover the A_1A_2BO, MNS and Rh groups of the Sikhs. As the work proceeded the rapid progress of research on the abnormal hæmoglobins (one of which is responsible for sickling) made it desirable to look for those as well. All blood samples were

TABLE 1

The A_1 A_2 BO blood groups of 213 Sikhs

Phenotype	Number observed	Frequency observed	Frequency expected	Number expected
O	69	*0·324*	*0·329*	70·1
A_1	37	*0·174*	*0·167*	35·5
A_2	11	*0·052*	*0·052*	11·2
B	79	*0·371*	*0·365*	77·8
A_1B	12	*0·056*	*0·064*	13·6
A_2B	5	*0·023*	*0·023*	4·9
Total	**213**	**1·000**	**1·000**	**213·1**

Gene frequencies

A_1	0·123
A_2	0·044
B	0·260
O	0·573
Total	1·000

TABLE 2

The ABO blood groups of 600 Sikhs *

Phenotype	Number observed	Frequency observed	Frequency expected	Number expected
O	205	*0·342*	*0·349*	209·2
A	152	*0·253*	*0·245*	147·1
B	199	*0·332*	*0·324*	194·2
AB	44	*0·073*	*0·083*	49·6
Total	**600**	**1·000**	**1·001**	**600·1**

* The 213 listed in table 1 are not included in these 600.

Gene frequencies

A	0·180
B	0·229
O	0·590
Total	0·999

tested with the sera anti-A, -B, -C, -D, -E, -c, -M, -N, -S. Samples positive with anti-A were tested with anti-A_1, and those positive with anti-E, with anti-e. Those which were negative with a saline anti-D were tested by means of the indirect anti-human-globulin technique

with several incomplete anti-D sera in order to detect the antigen D^u if present. The anti-C serum used had, as usual, the specificity anti-C+anti-C^w. Samples positive with this serum among the last 109 examined were further tested with pure anti-C^w. The same 109 samples were also tested with anti-Henshaw (all with negative results), and the hæmoglobin from them was tested at St Bartholomew's Hospital by means of paper electrophoresis. A further 170 specimens were tested electrophoretically but were not examined for blood groups. Samples from another 600 individuals were tested at Poona with anti-A and anti-B. The results of our tests are shown in tables 1-5. Gene frequency calculations were performed by the methods described by Mourant (1954).

The frequencies of the A, B and O genes fall well within the range

TABLE 3

The MNS blood groups of the Sikhs

Phenotype	Number observed	Frequency observed	Frequency expected	Number expected
MMS . . .	50	*0·235*	*0·251*	53·5
MsMs . . .	39	*0·183*	*0·159*	34·0
MNS . . .	51	*0·239*	*0·221*	47·1
MsNs . . .	44	*0·207*	*0·239*	50·9
NNS . . .	8	*0·038*	*0·039*	8·4
NsNs . . .	21	*0·099*	*0·090*	19·1
Total . .	213	1·001	0·999	213·0

Chromosome frequencies

MS	0·242
Ms	0·399
NS	0·060
Ns	0·299
Total . . .	1·000

of frequencies characteristic of north Indian populations. The A_2 gene is present; its frequency appears higher than in most north Indian populations, but the difference is not statistically significant. The MNS frequencies also are similar to the values previously found for the region but the frequency of the Ms chromosome is among the higher reported values.

The Rh frequencies also are fairly typical of the north Indian region, but with the frequency of chromosomes CDe and cDE among the higher of the values previously reported, and that of cde among the lower. The C^w gene had previously been found in the Indian sub-continent (in Pakistan) (Chaudhri *et al.*, 1952), only in the rare combination C^wde. In the present series, in the absence of the genotype C^wde/cde, it is presumed that, as in Europe, C^w occurs in the combination C^wDe. The typically African cDe chromosome

TABLE 4

The Rh blood groups of the Sikhs

Phenotype	Number observed	Frequency observed	Frequency expected	Number expected
CCDee *	91·636	*0·430*	*0·423*	90·2
CCD^{u}ee	1	*0·005*	*0·001*	0·3
C^{w}CDee *	4·364	*0·020*	*0·019*	4·1
CCddee	0	...	*0·000*	0·0
CcDEe	30	*0·141*	*0·154*	32·7
C^{w}cDEe	0	...	*0·003*	0·7
CcDee *	57·156	*0·268*	*0·266*	56·6
CcD^{u}ee	0	...	*0·010*	2·2
C^{w}cDee *	1·844	*0·009*	*0·006*	1·3
Ccddee	1	*0·005*	*0·005*	1·0
ccDEE	5	*0·023*	*0·014*	3·0
ccDEe	9	*0·042*	*0·051*	10·8
ccDee	3	*0·014*	*0·012*	2·5
ccD^{u}ee	1	*0·005*	*0·004*	0·8
ccddee	8	*0·038*	*0·031*	6·6
Total	**213·000**	**1·000**	**0·999**	**212·8**

* For the first 104 specimens tested, no distinction was made between C and C^{w}. It has therefore been assumed that the phenotypes CCDee and CcDee among these specimens included C^{w}CDee and C^{w}cDee respectively in the same proportions as for the 109 specimens tested subsequently.

Chromosome frequencies

CDe	0·612
C^{w}De	0·015
CD^{u}e	0·027
Cde	0·013
cDE	0·118
cDe	0·029
cD^{u}e	0·011
cde	0·176
Total	1·001

TABLE 5

The hæmoglobins of the Sikhs

Genotypes	Numbers observed	Frequencies observed	Frequencies expected	Numbers expected
AA	274	*0·9821*	*0·9786*	273·03
AD	4	*0·0143*	*0·0213*	5·94
DD	1	*0·0036*	*0·0001*	0·03
Total	**279**	**1·0000**	**1·0000**	**279·00**

Gene frequencies

A	0·9892
D	0·0108
Total	1·0000

has a frequency below that usually found in the Mediterranean area, or even in northern Europe. The cD^{u}e chromosome, recognisable in one individual, is almost completely absent in northern Europe but is found, with very much higher frequencies than in the present case, in certain Mediterranean and African populations.

Five persons out of 279 were found to carry hæmoglobin D. Four of these had hæmoglobins A and D : one had only D and is presumed to be homozygous for the corresponding gene, but this conclusion must be accepted with reservations until confirmed by family investigations. It is clear, however, that hæmoglobin D, though not common, is something more than a sporadic mutant as had been suspected when it had been found only in one Sikh (Bird, Lehmann and Mourant, 1955) and in two families of European ancestry. At the same time as the tests on the Sikhs, specimens from 13 Punjabi Hindus were tested, with the discovery of one individual heterozygous for hæmoglobins A and D. The production of hæmoglobin D thus seems to be essentially a Caucasoid character. Hæmoglobin S, typical of Africans and of Indian and Arabian Veddoids, was not found, nor was hæmoglobin E, typical of S.E. Asia but penetrating into India.

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Note.—The present addresses of the authors are :—G. W. G. Bird : Armed Forces Medical College, Poona 1, India. H. Lehmann : St Bartholomew's Hospital, London E.1.

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REVIEWS

THE NUMBERS OF MAN AND ANIMALS. J. B. Cragg and N. W. Pirie, F.R.S. 1955. Edinburgh : Oliver and Boyd. Pp. i-vii, 1-152. 15s.

This book is a record of the Symposium held on 24th and 25th September 1954, by the Institute of Biology. Several aspects of the control and biological importance of population size are considered. Particular emphasis is given to the human population and to the dangers of its present rate of increase.

The book is a short reference work rather than a treatise on populations. Although the speakers were well chosen, there is no one theme running through the work. Instead there are a number of separate short stories with no very close connection between them. One point of agreement seems to be that the size of the human population must be controlled in the near future. Little is known concerning the factors which regulate the numbers of animals (and presumably plants). For Man, the limiting factors suggested were famine, disease and war. However, Chitty in his contribution indicated that in some animals there may be a decrease in fertility and length of life arising from physiological derangements in overcrowded conditions. Unfortunately, except for Penroses's paper on Man, the bearing of genetics on population dynamics is relegated to the discussions. The book is well edited, the most serious criticism being that many papers lack useful summaries.

The book contains no new or startling conclusions. It is, however, a convenient survey of some aspects of population dynamics and should be read and consulted by those concerned with the ecology, and the welfare and future of mankind.

P. M. SHEPPARD.

TRAITÉ DE GÉNÉTIQUE. Tome 1. Le mécanisme de l'hérédité. Génétique formelle. Tome 2. La génétique des populations. By Ph. L'Héritier. Presses Universitaires de France. 1954. Pp. 518. 2.400 fr.

For the reviewer L'Héritier's two volumes have the usual easy charm of French books. The first volume is a workmanlike survey of modern genetical concepts as relating to the higher plants and animals. After a short historical introduction (by a lapse of the pen Johannsen acquires Swedish nationality) an outline is given of Mendel's Laws in their modern form. The quantitative aspects of linkage are treated much better than in most textbooks. Two chapters are then devoted to chromosome mechanics, aberrations and variations of chromosome number, leading to a discussion of hybridity, and chromosomal taxonomy. The last chapter deals with mutations, mutagenic agents and the physical nature of the gene.

The second volume is a fairly elementary introduction to the mathematical aspects of population genetics in the sense of the theory of natural selection. The inbreeding coefficient is introduced, but we are also given the matrix method of determining the speed of approach to homozygosity in a system of regular inbreeding. The effects of selection, mutation, and assortative mating are derived. A good deal of the discussion is based on Wright's equation $\Delta p = pqdw/2\bar{w}dp$. However, L'Héritier gives an original discussion of the case of selection on a totally X-linked gene. Also he constructs an example (which seems to be inspired by the fate of the dinosaurs) to show how, *ceteris paribus*, natural selection could carry a species to extinction. The process requires a gene which gives superiority in

mortal combat but diminishes fertility. Some experimental researches on natural selection are noted but not described in detail. Genetic drift is discussed at some length. Fisher's proof of the ultimate extinction of a gene of neutral survival value is sketched and his result for the chance of survival in a finite population of an advantageous gene is given without developing the theory of gene diffusion. Otherwise Sewall Wright's methods are used. The book ends with a brief exposition of the Neo-Darwinian view of the origin of species and races. Taken in all this book is distinctly easier to follow than Malecot's, being in this respect similar to C. C. Li's though restricted to a smaller range of mathematical topics. It should provide French readers with a quite stimulating introduction to evolutionary theory and the mathematical challenge which that theory presents.

A. R. G. OWEN.

THE MECHANISM OF EVOLUTION. By W. H. Dowdeswell. Heinemann. 1955. Pp. ix+99. 6s.

The Scholarship Series aims to offer " lengthy essays on selected topics not adequately treated in textbooks " and which are still rapidly advancing. Mr Dowdeswell's book has the distinction which we would expect from its author and can be read with pleasure and profit by an audience much senior to that for which it is primarily intended. The writing is clear, the examples fascinating and the development of the argument is skilfully carried through.

After a brief account of Lamarck's speculations, he sketches Darwin's views on the efficacy of natural selection operating on variation and shows how Darwin was led to postulate a high rate of induced mutation in order to extricate himself from the difficulty into which he was led by a belief in blending inheritance. Dowdeswell then defines the programme which Neo-Darwinism sets itself, as consisting in the determination of the sources of variation, and a quantitative approach to the study of natural selection as happening now. Assuming Mendel's Laws he reviews genetic variation in many aspects and leads to the conclusion that normally we have opportunity to observe only micro-evolutionary change. He next considers natural selection and adaptation, with examples from modern field work in many different species.

Mr Dowdeswell is concerned also with showing how such field studies can be carried out with profit to evolutionary science by schools or other groups of natural historians. He stresses the importance of design if the data are to be precisely evaluated. Data on dispersal, survival rates and population size are particularly important, and he sketches various methods elaborated by the distinguished group of investigators to which he belongs.

A. R. G. OWEN.

DAS LEBEN DER GEWÄCHSE, EIN LEHRBUCH DER BOTANIK. Band I: Die Pflanze als Individuum. Fr. Oehlkers. Berlin: Springer. 1956. Pp. 463, 523 text figures. DM. 39.60.

This first volume considers the plant as an individual as opposed to the plant in the world to which the second volume will be devoted. The planning of the book goes back, according to the author, to 1935. In fact it seems to go back much further. Its proportions are surprisingly little changed to accommodate the new learning to which the author has for

over thirty years continually and largely contributed. It is startling to find the vast text of descriptive systematic morphology interrupted by condensed notes on the principles of genetics and physiology. These notes, we observe, even when as on p. 343 they are " ausserordentlich bedeutungsvoll ", have to appear in the small type which breaks the continuity of every page. The instructive series of photographs and diagrams of mitosis and meiosis which would have improved any textbook of genetics or cytology are here separated from all that is relevant and buried between phyllotaxy and flagellates. The same applies to the chemistry of respiration or pigmentation. The list of chromosome numbers is surprising from a leading cytologist for, although they are only slightly incorrect, their effect as a whole is misleading. It is painful to see so much good material losing its value by its proportions and arrangement.

C. D. DARLINGTON.

PORTRAITS FROM MEMORY. R. B. Goldschmidt. University of Washington Press, Seattle. July 1956. Pp. 181. $3.50.

In the rapid, or we may say hurried, development of science during the last 100 years the records of the men who have been responsible have been treated with too little respect. The destruction of Darwin's manuscripts is typical of the loss we have suffered. In these circumstances Professor Goldschmidt has done a service in rescuing from oblivion his recollections of the men who made the richest period of German science. He describes what he rightly calls the Heroes of German Zoology, the men who worked out the meaning of the cell, the functions of the nucleus and also the pattern of animal development. They worked mostly in the universities of the recently independent south German states enjoying, between 1871 and 1914, the prosperity of the new empire.

As sometimes happens the author has in this case tended to underestimate the importance of his subject. In dealing with the contributions of some workers he has been explicit but with others quite vague. He whets our appetite without by any means satisfying it. Yet there is a danger that the large published works of 70 or 80 years ago to which Goldschmidt refers as " famous " or " classical " may become " once famous " and thus indeed cease to be classical. Within this great bulk are concealed the origins of the chromosomes theory, of the notions of the gene, of segregation and of crossing over, origins future generations will labour greatly to discover.

A few small difficulties are worth pointing out. Kammerer surely did not reveal his pathological mind by committing suicide. By doing so five weeks after his exposure he showed himself relatively normal ; much more so than when, for example, he invented the story of reciprocal crosses with reciprocal ratios (3 : 1 and 1 : 3) in the F_1. What Goldschmidt says is a valuable addition to the study of this episode and of scientific fraud in general. It is a study which, unfortunately, becomes not less but more important as science grows and flourishes.

Again, English readers may be distracted by some uses of words. Professor Goldschmidt is writing for an American audience. " Professor " therefore often means any university teacher. " Protector " is used for patron, " sanitation " for disinfestation, " auditing " for attending a class. " Anglo-Saxon " is also to be taken in a derivative sense.

This little book will be indispensable for the historian of science. Biologists in all countries will also be grateful to Professor Goldschmidt for it. They will ask for more. C. D. DARLINGTON.

THEORETICAL GENETICS. R. B. Goldschmidt. University of California Press. 1956. Pp. 563. 64s. net.

No-one is better qualified by his experience than Professor Goldschmidt to introduce us to genetic theories. He is the most learned of modern geneticists. He was brought up in the German school of cytology on whose work so much of our present genetics is founded or based. He has practised experimental breeding for nearly fifty years using a variety of animals and a variety of techniques. His views are too complex to allow of any charge of mechanistic optimism or philosophic naïveté to be advanced against them such as has been advanced against previous revelations in this field.

Goldschmidt's writings have given us many indications of what he is likely to say on this subject. Like his European contemporaries he knows too much ever to have swallowed the chromosome theory of heredity in its purest form. He will reject the billiard-ball gene and the cytoplasmic vacuum. He will emphasise continuity and integration and interaction and development at the expense of discontinuity and preformation. He will reject many specious and widely held assumptions and generalisations. And he will keep the discussion on a high theoretical level only stooping where necessary to pick up the evidence.

All this Professor Goldschmidt certainly attempts to do. He points out that he is going to omit wide ranges, especially the evolutionary ranges, of the subject which do not seem to him to matter for his present purpose. What does matter?

Goldschmidt is concerned almost entirely with gene structure and gene action. And in this field he is concerned above all with demolishing the classical idea of a corpuscular gene. Clearly the way in which we divide the genetic materials into units is crucial for all parts of the theory of genetics. Clearly also the introduction of new techniques has made it possible to see these units in many different ways. According to whether we are discussing chemical structure or mutation or crossing over and segregation the units seem to have different dimensions and relations. The same kind of difference arises when we turn from the inbred line to the natural population or the complex heterozygote species; or from the result of development to the process of development.

To most geneticists at the present day these differences are accepted (one would suppose) as inherent in the nature of the materials concerned. To be sure when they are accepted they demand adjustments and qualifications of language. These transformations require hard thinking but we see no inconsistency in accepting them. Not so Professor Goldschmidt. To him the old and the new appear inconsistent and to deny their inconsistency is to fail to see the truth and the light.

Professor Goldschmidt himself however is not always willing to see the light if a flicker of doubt will assist him. And flickering abounds in the observation in the cell. Let us glance at the chromomeres in polytene nuclei. In them, as in meiotic nuclei, we find indications of a unit of integration in physiological and perhaps also in genetic activity. We find

indications also that this unit can change its hierarchical value, since it may split in the course of development. Again in the observation of chromosomes we discover that some genes are made up by a repetition of similar parts whose supplementary action is responsible for the organisation of the spindle and of the nucleolus. We also discover that canalised interactions sometimes take place along great lengths of chromosome ; sometimes they are inhibited from doing so ; and sometimes they alternate cyclically in these interactions during mitosis.

This type of inference, which would have helped Goldschmidt's argument in some ways and would have hindered it in others, he overlooks. Another type of inference necessary to an integrated theory of the cell as the agent of development, of heredity, and even of infection, Goldschmidt does not overlook : he discusses it again and again. This is the inference of cytoplasmic heredity. He examines the observations of Lwoff on fibre-forming genetic particles in the protozoa. He describes the experiments of Lederburg and others on the transfer or recombination of genetic particles as phages among bacteria. He notices the conclusions of Bauer and Renner and Schwemmle on the genetic continuity and individuality of plastids. He has something to say about the genoids of l'Héritier. He even refers to his own work on *Lymantria*. Nevertheless he cannot bring himself to believe that there are genetic particles in the cytoplasm ; and, if there are, he begs us not to call them plasmagenes. " Why not," he asks (p. 197), " simply speak of self-duplicating cytoplasmic elements of different types ? " The answer is that some writers wish to speak simply. And those who have most to say sometimes wish to be brief.

Professor Goldschmidt's difficulties with the cytoplasm arise from many sources. One is that he has omitted to notice the work of recent years on plasmagenes and on viruses in plants. He therefore has not realised the diversity of relations that exist among such particles or between them and the nucleus. His definitions—for presumably he has definitions at the back of his mind—are not therefore adjusted to the facts as we now know them. Particles in the cytoplasm, subject to the influence of the nucleus, can change their activities without changing their character ; and they can also change their activities and their character together ; in other words, they can mutate. Failing to recognise these circumstances Goldschmidt naturally has not enquired further about such particles or such situations. He thinks that self-propagating particles are either " spontaneous " and " totally gene-independent " in their activities or, on the other hand, entirely subordinate and therefore of no genetic significance (pp. 236, 242). The possibility of interaction and mutual control, the idea of what we may call a constitutional government, which he is so ready to admit between genes within the nucleus, does not enter into his argument. Is it too simple a notion perhaps to be conceived as applying to unknown molecular systems ? We cannot tell. We merely observe an inhibition.

Inhibited, it seems, by the same fear of the obvious, is Goldschmidt's account of the relations of viruses and nuclear genes. A large part of his book is devoted to explaining to us the hierarchical organisation of the nuclear gene. So complex is it that we ought indeed no longer to use the term at all (p. 157).

Yet, when he discovers that viruses " have a complicated structure

permitting mutation, genetic recombination, linkage and even linkage groups", he concludes: "Thus a comparison with a gene is ruled out" (p. 244).

In reaching this conclusion Goldschmidt is thinking, of course, not of the evidence but of another notion of his own, namely the notion that all gene mutation is by rearrangement (p. 141). This view is mystical in the same sense as Lotsy's idea that all evolution was due to hybridisation. It can, to be sure, be reconciled with genetic materials constructed out of varying arrangements of four nucleotides. But can it be reconciled with a "hierarchy of organisational (molecular) patterns in the chromosome" (p. 487)? Surely not. For if we have both a pattern and a hierarchy the particles that are being rearranged must be of a higher order than nucleotides. At least two levels of rearrangement must be used, nucleotide and polynucleotide. Rearrangement is not then the simple formula that Goldschmidt supposes. We are in fact back with the distinction between intra-genic and inter-genic change. This is a provisional and crude distinction; but it is not a specious distinction since it admits that our knowledge is provisional and crude. Why indeed pretend otherwise?

If Professor Goldschmidt contradicts himself occasionally it must be admitted that he confirms himself far more often. In this book the repetition and cross-referencing are formidable. The repetitions, to be sure, shift their emphasis from time to time. The qualifications of statements by parenthetic words, phrases and whole sentences interpolated in other sentences make the style difficult and sometimes ambiguous. Worse still, such phrases as "I like to think" and "it is generally accepted" are ingratiating gestures but out of place in a work on theoretical genetics. They offend us in the treatment of a subject where rigorous argument is always needed and has sometimes been achieved.

Is it then possible to discover a continuous argument, a guiding principle or an original and individual view in this book? Professor Goldschmidt's approach to scientific theory is indeed of a recognisable type. He believes in "synthesis", that is, a new synthesis. He believes the facts are known on which he can base such a synthesis. Indeed, he believes many of them have been known for some time since he will refer to concepts which are "historical" as an argument for accepting them as a part of his synthesis (p. 196). These beliefs are an illusion. They lead, in Goldschmidt's treatment, to a short-circuiting of the tedious processes of scientific induction. How this happens we see in the text. His hypotheses arise he says "logically" (p. 488). How true! They arise by deduction from certain great *a priori* ideas. These ideas become doctrines. As he frequently tells us, what he says now he has repeatedly said before. The repetition is expected to carry conviction.

What are these doctrines? The chief of them seems to be, as perhaps it has to be, a doctrine of uniformity. "All the regularities of genetics and cytogenetics" he says (p. 272) compel us to reject certain evidence of gene action. But unfortunately Goldschmidt has chosen the wrong regularities. The regularities of chromosome organisation as we observe them in the cell are not those concerned with gene action. Heterochromatin, nucleolar organisation, genic balance, diploidy, stability of chromosome numbers in development or in the population, these are concerned with gene action. And they are all properties in which groups of organisms

radically differ. Again (p. 483) we are told that " the organisational differences, say between Diptera, Orthoptera and Lepidoptera, are not of a magnitude that requires different cellular mechanisms ". But since they are known to have different cellular mechanisms (polytene nuclei only in Diptera, for example) why not note the fact? Why not accept it? And why not connect it with the observed differences in chromosome evolution?

These, it seems to the reviewer, are errors. They are not errors of fact but of judgment, of judgment in regard to the method of inference. In another kind of judgment, in the choice of materials and methods for experiment, Professor Goldschmidt has always been original and frequently successful. But in the choice of materials and methods for inference he shows himself laboriously unsuccessful. And this kind of judgment is the only stuff out of which we can make theoretical genetics.

C. D. DARLINGTON.

GENETICAL SOCIETY OF GREAT BRITAIN

ABSTRACTS of Papers read at the HUNDRED AND TWENTY-FIRST MEETING of the Society, held on 11th and 12th JULY 1956, in the DEPARTMENTS OF BOTANY AND ZOOLOGY, UNIVERSITY OF OXFORD

COMPARATIVE SENSITIVITY OF MITOTIC STAGES

D. DAVIDSON
Botany Department, University of Oxford

Chromosome breaks and reunions induced by X-rays were scored in $4x$ nuclei derived from $2x$ nuclei after colchicine treatment and it was shown that metaphase is the most sensitive stage.

Breaks per chromosome recovered at the following mitosis are more frequent in $4x$ nuclei after irradiation of prophase, metaphase or telophase than in $2x$ nuclei found in the same samples owing to the fact that the $2x$ nuclei are not accurately timed and include less sensitive resting stage samples. On the other hand, breakage is less frequent in $4x$ nuclei than in $2x$ nuclei after irradiation in resting stage. This may perhaps be due to a genuine physiological difference between the two types of nuclei.

THE CONTRIBUTION OF INDUSTRIAL MELANISM IN THE LEPIDOPTERA TO OUR KNOWLEDGE OF EVOLUTION

H. B. D. KETTLEWELL
Department of Zoology, University of Oxford

(Cinematography by Dr Niko Tinbergen, Department of Zoology, University of Oxford)

There are about seventy species of moth in this country which at the moment are in the process of changing their complicated wing patterns, built up for concealment by day on lichened tree trunks, boughs or rocks, to an all black coloration. This was first noted about 1840, and the rate of spread of the black forms constitutes the most striking evolutionary change ever witnessed in any living organism. It has been postulated that birds must be responsible for bringing about a differential selection according to the camouflage efficiency of the moths. Strangely, absolutely no evidence has been forthcoming that birds actually take and eat stationary insects sitting in their natural positions. In 1953, a series of mark-release-recapture experiments was conducted near the industrial area of Birmingham, where about 10 per cent. of the Peppered Moth (*Biston betularia* L.) are of the white form. This film records the techniques used in the experiments, including those for breeding a sufficient number of moths necessary for a large scale experiment of this sort. Moreover, it also provides a visual record of six species of birds predating the moths, firstly, in an industrial area and, secondly, by contrast, far outside in an unpolluted woodland in Dorset. Furthermore, it will be noted that the birds take them in their order of conspicuousness. The film does, in fact, show that birds act as responsible agents for the elimination of the white forms in polluted countryside and, conversely, of the black forms in unpolluted England. This single fact is sufficient to account for the spread of melanic forms throughout central and eastern England, quite apart from other character and behaviour differences which, undoubtedly, exist and which are at present the subject of investigation.

An introduction to, and a commentary on, the film will be given by Dr E. B. Ford.

METABOLIC AND SENSORY POLYMORPHISMS

A. C. ALLISON
Radcliffe Infirmary, Oxford

Examples of metabolic and sensory polymorphisms deserve consideration for several reasons, (i) they are likely to be commoner than the classical pigmentary and other types of polymorphism, and will eventually mark a number of loci in those species in which we are most in need of marker genes for studies of linkage—*e.g.* the larger domestic mammals and Man ; (ii) they illustrate the way in which the products of allelomorphic genes differ and throw light on problems of gene action and biochemical evolution ; (iii) it seems probable that the polymorphisms are maintained by several genetic mechanisms, some of which have not yet been illustrated by examples. Metabolic polymorphisms may also bridge the gap between the classical polymorphisms and polygenic inheritance.

In general, three types of metabolic polymorphisms are found :—(1) Those where allelomorphic genes give rise to distinct products, both of which are present in the heterozygote. Examples of this are to be found in the hæmoglobin types and milk protein types. (2) Those where the heterozygotes give rise to products different from those of either homozygote, *e.g.* some blood groups and human serum protein types. In some of these polymorphisms the selective forces leading to stable equilibria are known. (3) Those where one allelomorph only gives rise to a distinguishable product, which is present in smaller quantities in heterozygotes than in those homozygous for the allele. Examples of this are to be found in genes controlling the formation of atropine esterase (and possibly xanthophyllase) in rabbits, catalase in dogs, cyanogenic glucosides and enzymes hydrolysing them in clover, and enzymes methylating dithiolisobutyric acid and metabolising beet pigment in Man. The remarkable genes controlling cation concentrations in sheep erythrocytes should probably be included in this group also.

Some sensory polymorphisms are autosomal, *e.g.* inability to taste PTC or to smell certain flowers. Others are sex-linked recessive characters, *e.g.* colour blindness and inability to smell cyanides. Of the way in which these polymorphisms are maintained we are entirely ignorant.

THE CHROMOSOMES OF SOME PRIMARY AND TRANSPLANTED TUMOURS OF THE MOUSE

C. E. FORD, R. H. MOLE and J. L. HAMERTON
M.R.C. Radiobiological Research Unit, Harwell

Differences from the normal set of 40 chromosomes have been detected in many primary tumours of the mouse, most of which arose in irradiated animals. Both structural and numerical deviations are frequent and stem-line numbers of 41, 42 and 43 have been observed. Further changes have occurred in certain of these tumours after transplantation. Evidence that such changes may still take place many years after a transplanted tumour was first established has been obtained from the ascites tumour Sarcoma I. The significance to be attached to this chromosomal variation will be discussed.

VARIABLE POLLEN TETRADS IN THE STYPHELIEÆ

S. SMITH-WHITE
Botany Dept., University of Sydney

Three types of pollen are produced by species of the Styphelieæ. In some it is matured as tetrads, a character usual in the Ericales. More frequently it is monad, one microspore in each tetrad being functional. In four species the mature tetrads are variable—mature tetrads may contain 0, 1, 2, 3 or 4 functional pollen grains.

The monad type is evidently derived from the tetrad type, and must involve asymmetrical cytoplasmic differentiation within the mother cell. Acentral MI spindles, and migration of microspore nuclei are evidence of this cytoplasmic differentiation.

Variable tetrads may represent either a step in the origin of the monad pattern, or a breakdown of that type. Available evidence supports the latter view.

The immediate cause of tetrad variability may lie in the cytoplasm or in the microspore nuclei. In *Astroloma conostephioides*, meiotic asymmetry suggests an unstable cytoplasmic differentiation, and the tetrad type frequency distributions in the species may conform to this view. In *A. pinifolium*, asymmetry of meiosis is scarcely evident and the tetrad type frequency distributions favour the inference of chromosomal segregations, with duplicate systems of complementary lethals and strict second division reduction. In both species there is direct evidence of structural hybridity, including deficiencies, inversions and interchanges, but these must be regarded as supplementary rather than primary causes of tetrad segregation.

RIBBON CHROMOSOMES IN SNAIL SPERMATOZOIDS AS A DEMONSTRATION OF POLYNEMY

C. D. DARLINGTON
Botany Dept., University of Oxford

Electron microscope photographs of P.-P. Grassé and his collaborators (*C.R. Acad. Sci.*, Paris, 12th Mar. 1956) indicate a ribbon structure which I interpret as multiple (about 64) nucleoprotein columns. Urodeles should have higher, flies like *Drosophila* lower, degrees of polynemy.

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